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NOTICE TO CONTRIBUTORS

General

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A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

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Units of measurement should be placed in parentheses at the head of the column, not in the body of the table. Descriptive notes should be kept to a minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

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HYDRANGEA RINGSPOT VIRUS IN NEW ZEALAND

By A. D. THOMSON, Plant Diseases Division, Department of Scientific and Industrial Research, Lincoln.

(Received for publication, 25 July 1960)

Summary

Hydrangea ringspot virus was isolated from *Hydrangea* plants in New Zealand. Concentration of the virus by density-gradient centrifugation is described.

A virus has been transmitted by sap-inoculation methods from *Hydrangea* plants to *Gomphrena globosa* L. and *Chenopodium amaranticolor* Coste and Reyn. Local lesions were produced on both plants. No infection was obtained on *Nicotiana tabacum* L. (var. White Burley) or *Phaseolus vulgaris* L. (var. Prince). The virus gave a positive gel-diffusion test with antisera to an English isolate of hydrangea ringspot virus. This evidence, as well as the presence of rod-shaped particles in preparations from infected *Hydrangea* plants (Fig. 1) suggests that the New Zealand virus is closely related to the English hydrangea ringspot virus described by Hollings (1958). Hollings has made stability tests and described the symptoms produced by the English virus on a number of hosts.

Hydrangea ringspot virus was isolated from 15 *Hydrangea* plants (20 plants tested) growing at Lincoln, Papanui, Christchurch, Dunedin, and Roxburgh. Certain of these plants were obviously stunted and in one case leaves showed chlorotic ring lesions. There is no evidence for aphid transmission (Hollings, 1958). Tests for soil transmission are being made.

The virus was concentrated from infected *Hydrangea* leaves using density-gradient centrifugation. Sap expressed from frozen leaves was centrifuged at 5,000 G for 20 min. and the resulting supernatant fluid was then centrifuged at 30,000 r.p.m. for 60 min. in a Spinco model L preparative ultracentrifuge using the No. 40 rotor. The pellet was re-suspended in a small volume of water, centrifuged at 5,000 G for 10 min. and 0·2 ml of the concentrate

FIG. 1—Electron micrograph of rod-shaped particles from zone C; gold-shadowed. × 84,000

floated on a sucrose gradient prepared by layering 0·9 ml each of 40%, 30%, 20%, 10% (W/V) sucrose in plastic centrifuge tubes. The tubes were centrifuged at 25,000 r.p.m. for 60 min. in the S.W. 39 rotor. Two opalescent zones (Fig. 2, B and C) were observed in the tubes by light scattering. Most infectivity was present in zone C (Table 1) and this zone contained rod-shaped particles (Fig. 1) similar to those reported for hydrangea ringspot virus by Sisler *et al.* (1957) and Hollings (1958).

TABLE 1—Infectivity in Fractions Removed from Density-gradient Tubes Layered with Hydrangea Ringspot Virus

Zone*	Relative Infectivities†	Zone*	Relative Infectivities†
A	2	C	45
B	11	D	4

*See Fig. 1. Zones removed by puncturing the centrifuge tube wall with a hypodermic needle. Samples dialysed against water at 20° for 24 hours before infectivity tests were made.

†Mean number lesions on 6 leaves of *Gomphrena globosa.*

These tests provide further evidence to that obtained by Sisler *et al.* (1957) from electrophoretic studies for the association of infectivity with the rod-shaped particles. Zone B (Fig. 2) may contain "anomalous" virus protein or microsomal material. Two components with different electrophoretic mobilities were described by Sisler *et al.* (1957) in their preparations of hydrangea ringspot virus.

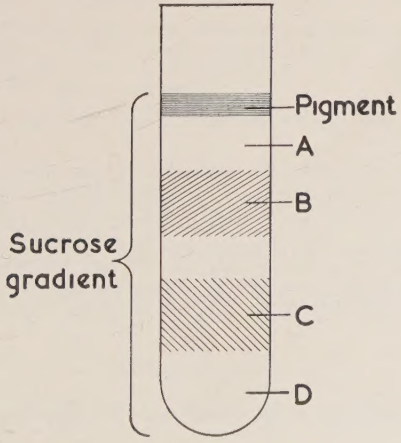

FIG. 2—Diagram of opalescent zones in centrifuge tube after centrifuging hydrangea ringspot virus in a sucrose gradient.

ACKNOWLEDGMENTS

The author's gratitude is expressed to Professor J. A. R. Miles, University of Otago, for the use of a Spinco model L ultracentrifuge, also to Mr M. Reynolds, University of Otago, who took the electron micrographs, to Mr M. Hollings, Glasshouse Crops Research Institute, Littlehampton, England, who made the serological tests, and to Mr C. J. Miles, Crop Research Division, for the photographs.

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HOLLINGS, M. 1958: Hydrangea Ringspot in Britain. *J. hort. Sci. 33*: 275–281.

SISLER, H. D.; CHANG, S. C.; REAGAN, R. L.; and BRIERLEY, P. 1957: Purification and Properties of Hydrangea Ringspot Virus. *Phytopathology 47*: 491–494.

MECHANISMS OF ION ADSORPTION BY INORGANIC SOIL COLLOIDS

By M. FIELDES, Soil Bureau, Department of Scientific and Industrial Research, Wellington, and R. K. SCHOFIELD, Department of Agriculture, University of Oxford.

(Received for publication, 23 August 1960)

Summary

Net electric charges on soil clays at different pH values in the range 3 to 8 have been determined from measurement of sodium and chloride retentions.

Taita soil clay (from greywacke) containing the micaceous clay illite and metahalloysite had dominantly negative net charge throughout the pH range with an increase between pH 6 and 7 which was small relative to the total charge. This result is similar to published results for Rothamsted soil (illitic), montmorillonite, and other micaceous clays.

On the other hand, Taupo and Egmont soil clays (from volcanic ash) containing allophane had positive net charge below pH 5·5, but acquired considerable negative charge between pH 6 and 7; thus they had electric charge which may be described as dominantly variable.

Whatatiri soil clay (from basalt) containing metahalloysite and gibbsite behaved like allophane but the amounts of charge were less.

Permanent negative charge of clays has been attributed to isomorphous substitution. In micaceous materials, considerable permanent negative charge is due to isomorphous substitution in octahedral positions. In allophane, because of its origin from glasses and feldspars, octahedral positions are lacking and its characteristic variable charge is here attributed to the behaviour of specific sites originating from broken bonds of aluminium in tetrahedral coordination. Montmorillonite and other similar clays have a small amount of variable charge which is probably similar in origin.

This concept of tetrahedral aluminium explains satisfactorily apparently for the first time (a) reversible loss of water from allophane above 105°C (b) increase of cation retention with increasing pH, and (c) the mechanism of anion retention and the order of affinity of soil clays for different anions.

The nature of other possible specific sites originating from broken bonds at soil clay surfaces is outlined.

The tetrahedral aluminium site is considered in relation to its effect on soil structure, phosphate and molybdate fixation, and other characteristic properties of soils containing allophane. Similar properties are expected from hydrated feldspars.

Net charge at pH 5 is a measure of permanent negative charge while the difference between net charges at pH 5 and 7·5 represents amount of variable charge. Similar values for permanent and variable charge of the soil-clays examined were provided from titration curves which may be obtained more readily than the net charge results.

INTRODUCTION

Many soils from volcanic ash and basalt have distinctive properties which include:

(1) Well-developed soil structures

(2) Friability

(3) Low $SiO_2 : R_2O_3$ ratio of clay

(4) High phosphate-fixing power

(5) High pH, near 6, at low base saturation

(6) Clay fractions containing amorphous material, including hydrous oxides, allophane, and palagonite

(7) Mineral colloids with high cation-exchange capacity not due to montmorillonite

In an attempt to understand the relation of the nature of the colloids to the soil properties it was decided to investigate the possibility of characteristic activity of specific sites on the colloids.

MATERIALS

Nature of Samples

Samples selected for examination were:

SB. 6673: Subsoil of Taupo sandy silt, a yellow-brown pumice soil (genetic class 13a) from rhyolitic ash.

SB. 6674: Subsoil of Egmont brown loam, a yellow-brown loam (genetic class 14a) from andesitic ash.

SB. 6676: Subsoil of Whatatiri clay loam, a brown loam (genetic class 16a) from basalt.

SB. 6675: Subsoil of Taita clay loam, a yellow-brown earth (genetic class 5C) from greywacke.

Genetic class Nos are from Taylor (1948).

Taupo and Egmont soils contain allophane-type colloids, the Whatatiri soil clay contains metahalloysite, hydrous oxides, and amorphous matter derived from basalt, while the clay of the Taita soil, which was included for comparison, contains largely layer-type clay minerals.

METHOD

Tests were carried out on clay fractions less than 2μ. Soils were dispersed in water made to pH 10·8 with NaOH and clays removed by repeated sedimentation. Dispersed clays were flocculated at pH 4 (pH 3·2 was needed for Taita sample) with HCl. The aqueous material was removed and the clays were dried at below 60°C. Mineralogy of the clays is shown in Table 1.

TABLE 1—Soil Type and Mineralogy of Soil Clays Examined

Sample	Type Name	Clay % on Soil	Constituents of Clay
S.B. 6673	Taupo	4·9	Allophane AB.*
S.B. 6674	Egmont	12·7	Allophane,* lepidocrocite.
S.B. 6676	Whatatiri	77·9	Metahalloysite, gibbsite.
S.B. 6675	Taita	38·3	Illite, metahalloysite.

*Fieldes (1955).

The clays contain some unidentified amorphous material. In the Whatatiri sample, for instance, thermal analysis showed that gibbsite and metahalloysite did not account for all of the colloid. The clays contain also small amounts of sodium chloride from entrained solution. This was mostly water-soluble and could all be removed by 0·2 N KNO_3. The amounts of sodium and chloride removed were almost equal. The samples thus approximate to H-clays.

Electric Charge

Electric charges carried by the clays at different pHs were determined by a modification of a method used by Schofield (1949). The uptake of sodium and chloride from 0·2 N NaCl at different pHs was determined by leaching 5g of clay mixed with 5g air-dry silica sand on a sintered glass filter (Pyrex SF 3A/4) coated with gooch asbestos. The purpose of the asbestos was to prevent clogging of the filter, to distribute the clay evenly and prevent channels developing through cracking. pH of leaching NaCl was adjusted with NaOH or HCl. Rate of pH change was very slow and continuous leaching was usually needed for several hours till pH of the leachate approached a steady desired value which was taken to be the steady value of the treated clay. Surplus liquid was then sucked off and the filter weighed to determine the weight of entrained leachate. The clay was then leached free of sodium and chloride with 0·2 N NH_4NO_3, pH 9. The total sodium and chloride determined in the NH_4NO_3 was corrected for the amount due to entrained NaCl solution, giving values of Na and Cl retained by the clay at that pH. The procedure was then repeated for other pH values. Blanks on the asbestos showed that its retention was small and served to check the Na and Cl content of leaching solutions.

Na was determined gravimetrically and by flame photometry, Cl was determined volumetrically and gravimetrically. Analytical accuracy was good but triplicate leachings of samples to the same pH were reproducible only to $\pm$ 2%. This reproducibility did not justify minor corrections to net charge values. Net charge is taken here as the difference between Na and Cl retentions.

Titration Curves

To 1g samples in 100 ml 0·2 N NaCl amounts of HCl or NaOH were added to give additions of from 20 me. of acid to 100 me. of base per 100g sample in steps of 20 me. per 100g. pH values were measured with glass electrode and calomel reference electrode after end-over-end shaking for suitable times.

RESULTS

Na and Cl retention and net electric charges on clay at different pHs are shown in Figs 1–4. Net charge values are also shown in Fig. 5 together with values for Rothamsted subsoil (Schofield, 1949) and montmorillonite for comparison. Results of titration curves are shown in Fig. 6.

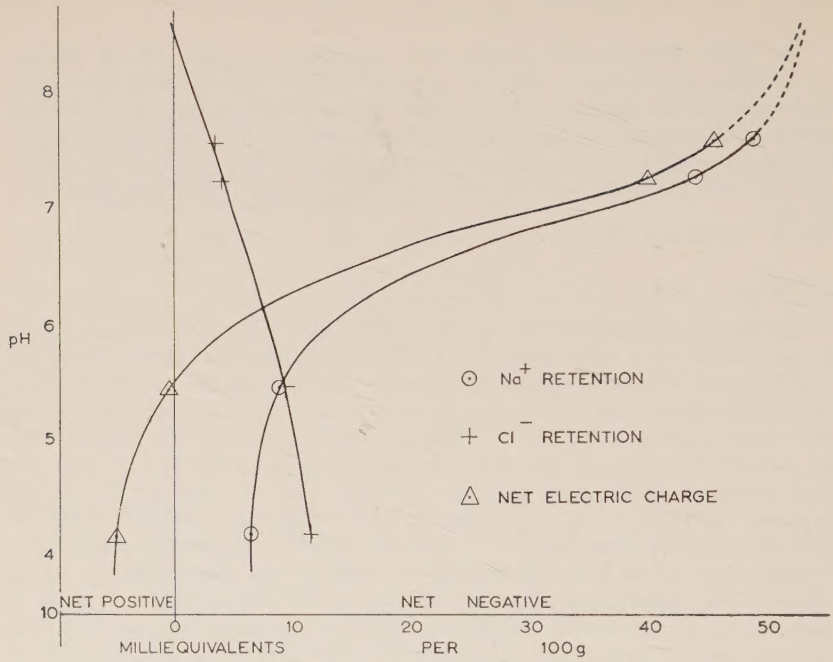


FIG. 1—Electric charges on Taupo soil clay in 0·2N NaCl at different pH.

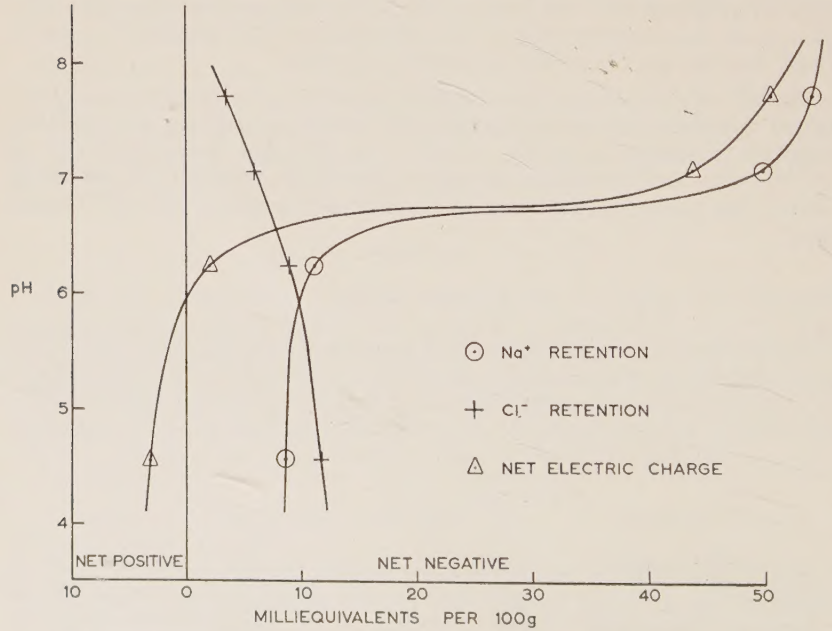


FIG. 2—Electric charges on Egmont soil clay in 0·2N NaCl at different pH.

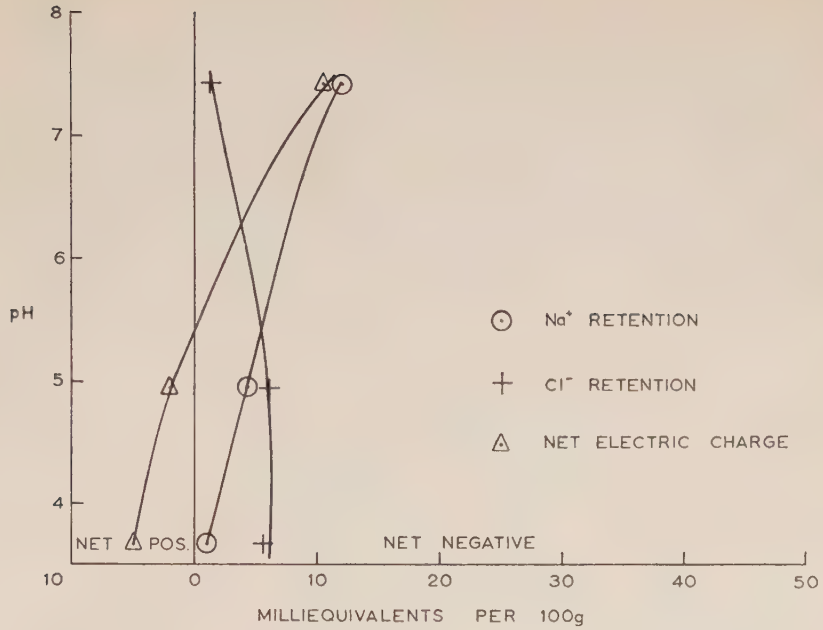


FIG. 3—Electric charges on Whatatiri soil clay in 0·2N NaCl at different pH.

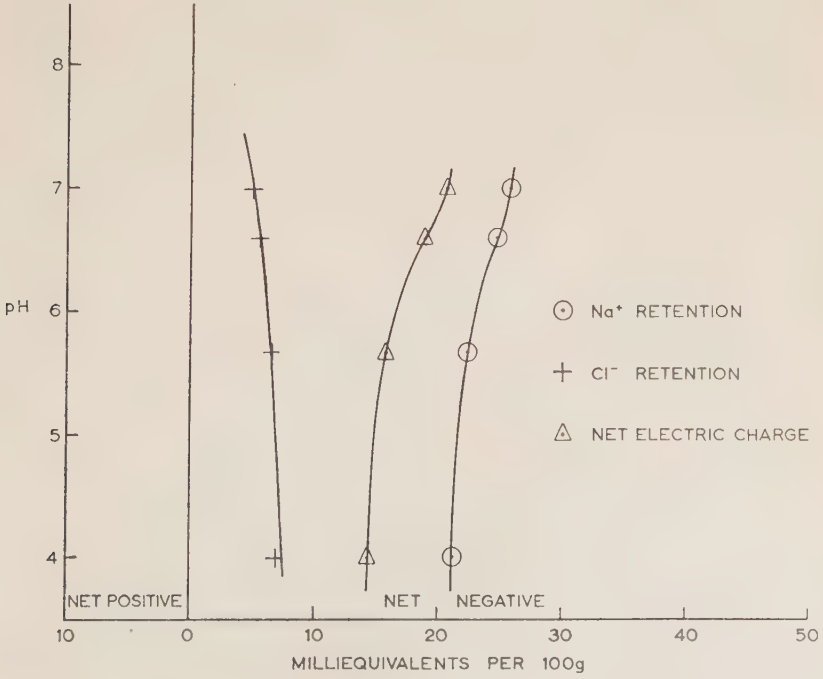


FIG. 4—Electric charges on Taita soil clay in 0·2N NaCl at different pH.

DISCUSSION OF RESULTS

Electric Charge

Results of Figs 1–5 illustrate the following points:

(1) Taita soil-clay which is illitic resembles the illitic Rothamsted subsoil in variation of net charge with pH (Fig. 5). Like montmorillonite,

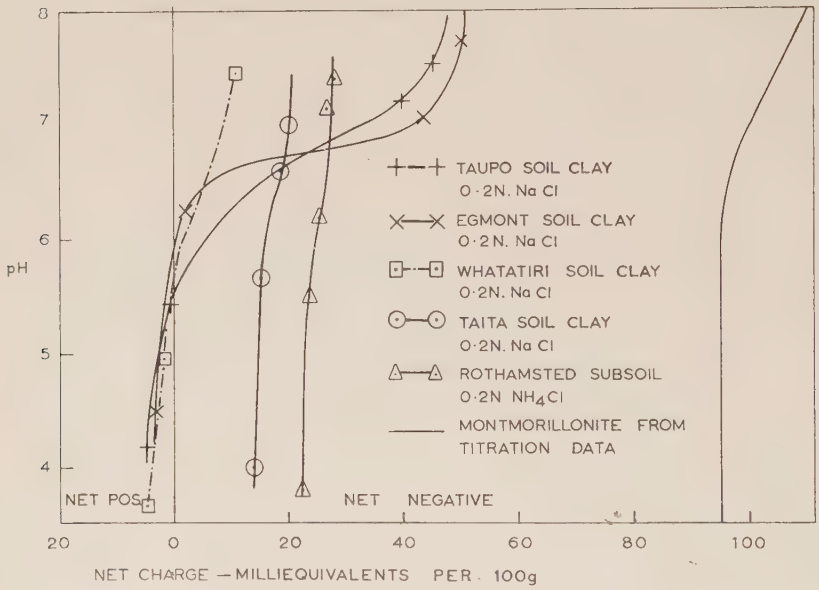


FIG. 5—Net electric charges on various materials at different pH.

both samples have net negative charge throughout pH range 4 to 7·5. Both increase slightly in negative charge between pH 4 and 6 with a more marked increase from pH 6 to 7·5. The increase of negative charge from pH 6 to 7·5 relative to total negative charge is greatest for Taita soil-clay, intermediate for Rothamsted subsoil and least for montmorillonite.

(2) Soil-clays from ash or basalt differ from the Taita soil clay and the other materials. They have net positive charge at pH below 5·5, an isoelectric point of zero charge between pH 5 and 6 and develop considerable negative charge between pH 5·5 and 7·5.

(3) Taita soil-clay has a permanent negative charge which may well be explained by isomorphous substitution in its illitic constituents similar to that in montmorillonite.

(4) Soil-clays from ash or basalt have negative charge which is dominantly variable.

(5) All soil-clays decrease in chloride retention steadily over the pH range 4 to 7·5. Sodium retention increases and chloride retention decreases for all samples but the increase in sodium retention has otherwise no evident relation to decrease in chloride retention. Above pH 5·5 increase in sodium retention exceeds decrease in chloride retention showing that new negative charges are developed on all soil-clays. This effect can be reproduced and appears to be reversible, indicating that variable negative charge is produced by changing pH on the exposed surfaces of all of the soil clays. Activation of specific sites is therefore a possibility.

Titration Curves

Titration curves of Figs 6 and 7 considered in conjunction with Figs 1 to 5 illustrate the following points:

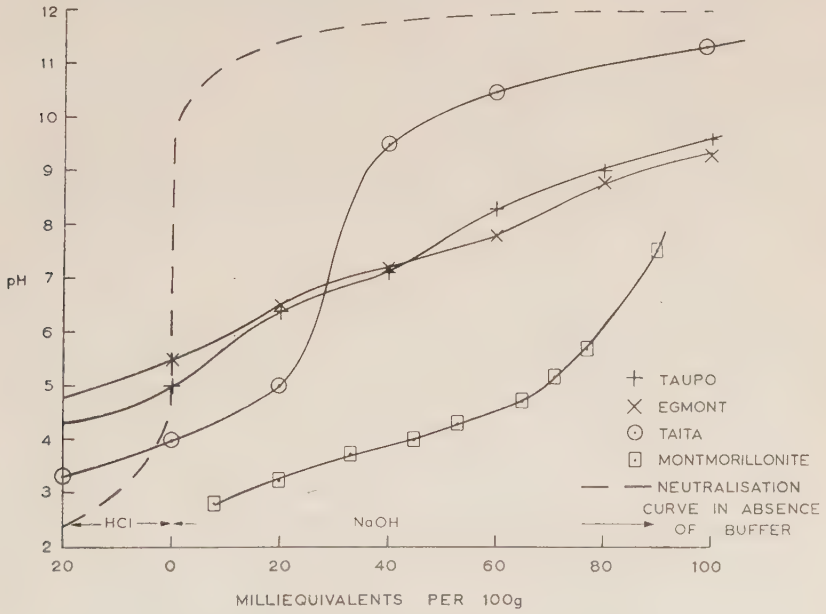


FIG. 6—Titration curves of clay in 0·2N NaCl.

(1) As shown in Fig. 4 at pH 4 Taita soil has 21 me. per 100g sodium retention (total negative charge) which changes only slightly at pH 5. According to Fig. 6 however, to reach pH 5 addition of 20 me. per 100g of NaOH is required, so that by pH 5 the total negative charge is almost neutralised. Most of the buffering of this soil is therefore below pH 5 (in 0·2 N NaCl). 20 me. per 100g of base raise the pH one unit (4 to 5) while only 10 me. per 100g more raise pH by

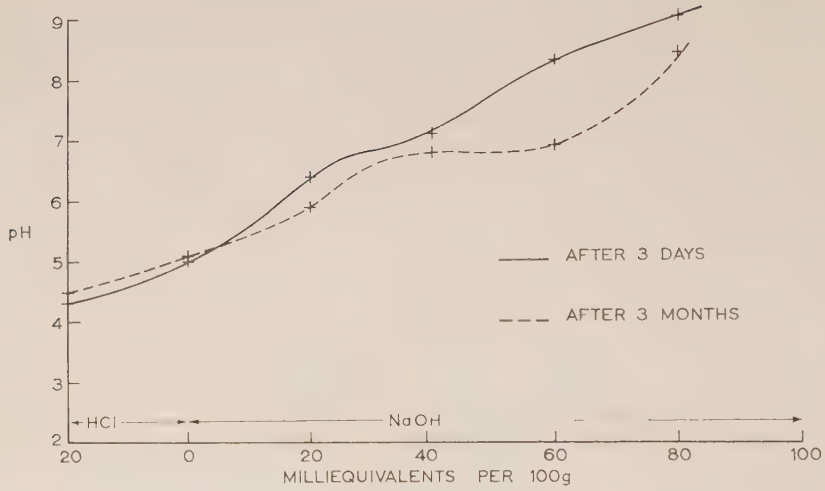


FIG. 7—Effect of time on titration curves of Taupo soil clay in 0·2N NaCl.

2·5 units (5 to 7·5). NaOH used below pH 5 is roughly a measure of total negative charge on the hydrogen clay. NaOH used above pH 5 is a measure of new negative charges developed by changing pH.

(2) For Taupo and Egmont soil-clays pHs of the hydrogen clays are at the pH of their isoelectric points pH 5 and 5·5 respectively (in 0·2 N NaCl). Above these pHs the amount of NaOH added appears to be roughly equivalent to the amount of new negative charge developed at the adjusted pH. Nearly 50 me. per 100g NaOH is required to raise the pH of these soil clays from near 5 to 7·5, compared with 10 me. per 100g for Taita soil-clay.

(3) On standing for three months pH values of Taupo (Fig. 7) and Egmont soil clays changed significantly towards the pH of their isoelectric points without bringing aluminium into solution. This effect appears due to equilibriation on difficultly accessible sites.

(4) Further meaning can be read into Figs 6 and 7, but perhaps the important point for the moment is that titration curves promise to provide a ready means of discriminating between permanent negative charge and variable negative charge on soils, and thus evaluate relative amounts of montmorillonite-like and allophane-like properties.

ORIGIN OF PERMANENT NEGATIVE CHARGE

Permanent negative charge capable of exchanging cations on montmorillonite is attributed to isomorphous substitution in octahedral positions. Other clay minerals with micaceous structures have permanent negative charge of similar origin.

Origin of Variable Negative Charge

Relation to Al

High variable negative charge is the distinctive property of allophane-type colloids of relatively high Al content. It is a property of the Taupo soil clay with low Fe_2O_3 content and thus it is not necessarily associated with Fe. Variable negative charge is also a feature of the Whatatiri soil-clay from basalt which is high in Al. It seemed possible that the effect might be related to Al particularly as artificially prepared hydrous alumina and alumino-silicates were known to have high cation-exchange capacities.

The effect of Al in artificial alumino-silicates was confirmed by Dr Kressman of the Permutit Co. Ltd., London (pers. comm.) who stated that (a) artificial alumino-silicates extensively manufactured for water softening before the advent of resins were made with high Si content if they had to withstand rigorous conditions such as boiling water, as high Si gave mechanical strength to the gel. For ordinary purposes high Al content was preferred as cation-exchange capacity increased with increasing Al; (b) that the form of cation retention curves with varying pH for artificial alumino-silicate was closely similar to the curves given by allophane-type colloids of Taupo and Egmont soil-clays which are also similar to the curves given by synthetic cation-exchange resins in which the cation-exchange is due to negative charge developed when the pH permits dissociation of a group, such as COOH, attached to the insoluble structure of the resin. The latter cases involve specific sites at the surface of the material.

Form of Al

Clay-size gibbsite which contains all of its Al in octahedral coordination has negligible negative charge at pH 7. It has no permanent charge because isomorphous substitution is low or absent. The absence of variable charge suggests that active sites on allophane if related to Al are not due to OH groups joined to Al in octahedral coordination.

Well formed acid-washed kaolinite, in which Al is octahedral, linked by O to tetrahedral Si, also has low permanent and variable charge, while montmorillonite, in which there is considerable isomorphous substitution, has high permanent negative charge, but also considerable variable charge. It seems from the case of gibbsite that sites causing variable negative charge are not connected with octahedral Al. This suggests that the variable charge might be connected with tetrahedral Al sites such as occur in montmorillonites and micaceous clay minerals in which Al may substitute extensively for Si in Si tetrahedra.

The implications of this with regard to allophane are obvious since this is formed in ash soils whose primary minerals are mainly feldspars and glasses in both of which Al is maintained in tetrahedral coordination by linkages through O with Si tetrahedra.

The suggestion therefore is: (a) that tetrahedral Al is the origin of active sites on allophane; (b) that allophane-like properties may originate

from precipitated alumino-silicate gel, from surfaces of particles of feldspar or glass and from Al in tetrahedral layers of micaceous clay-minerals; (c) since micaceous clay minerals, which may have up to one-third of their Si replaced by Al, do not have correspondingly great variable charge, the active sites due to tetrahedral Al must involve only those exposed at broken edges.

It therefore seems desirable to consider the nature of different types of sites which may be formed at broken edges of mineral structures containing Al.

External Sites Originating near Al

The linkages present in typical layer-type silicates in gibbsite and in feldspar are illustrated in Fig. 8. Consideration of these structures leads to the following possibilities for aluminium:

(1) In *gibbsite* aluminium may occupy only octahedral sites. There is little isomorphous substitution and this material has little permanent negative charge. Within its sheet structure all oxygens are similar and each oxygen is linked to two aluminiums and one hydrogen. Fracture of the structure occurs in the following way:

$$Al — OH — Al \rightarrow Al — OH^{-\frac{1}{2}} + {}^{+\frac{1}{2}}Al \text{ (Type 1 fracture).}$$

The breaking of two such bonds has the following result:

$$Al = (OH)_2 = Al \rightarrow Al — OH^0 + {}^0OH — Al \text{ (Type 2 fracture)}$$

The broken structure can thus achieve electrostatic neutrality. In the presence of water, oxygens of water molecules would tend to attach by coordination in the oxygen holes left in the octahedra but no ready dissociation would be expected. Little permanent charge and little variable charge should therefore be found on a pure gibbsite, as is the case.

(2) In well-formed *kaolinite* it is considered that aluminium occupies only the octahedral positions and there is little isomorphous substitution in either octahedra or tetrahedra so that permanent negative charge is small. Within its sheet structure there are four types of oxygens. Fracture of the structure gives rise to the following possibilities:

$$Al — OH — Al \rightarrow Al — OH^{-\frac{1}{2}} + {}^{+\frac{1}{2}}Al \text{ (Type 1 fracture)}$$

$$Al = (OH)_2 = Al \rightarrow Al — OH^0 + {}^0OH — Al \text{ (Type 2 fracture)}$$

$$Al — OSi — Al \rightarrow Al — OSi^{-\frac{1}{2}} + {}^{+\frac{1}{2}}Al \text{ (Type 3 fracture).}$$

$$Si — O — Si \rightarrow Si — O^{-1} + {}^{+1}Si \text{ (Type 4 fracture)}$$

$$Al — O — Si \rightarrow Al — O^{-1} + {}^{+1}Si \text{ (Type 5 fracture)}$$

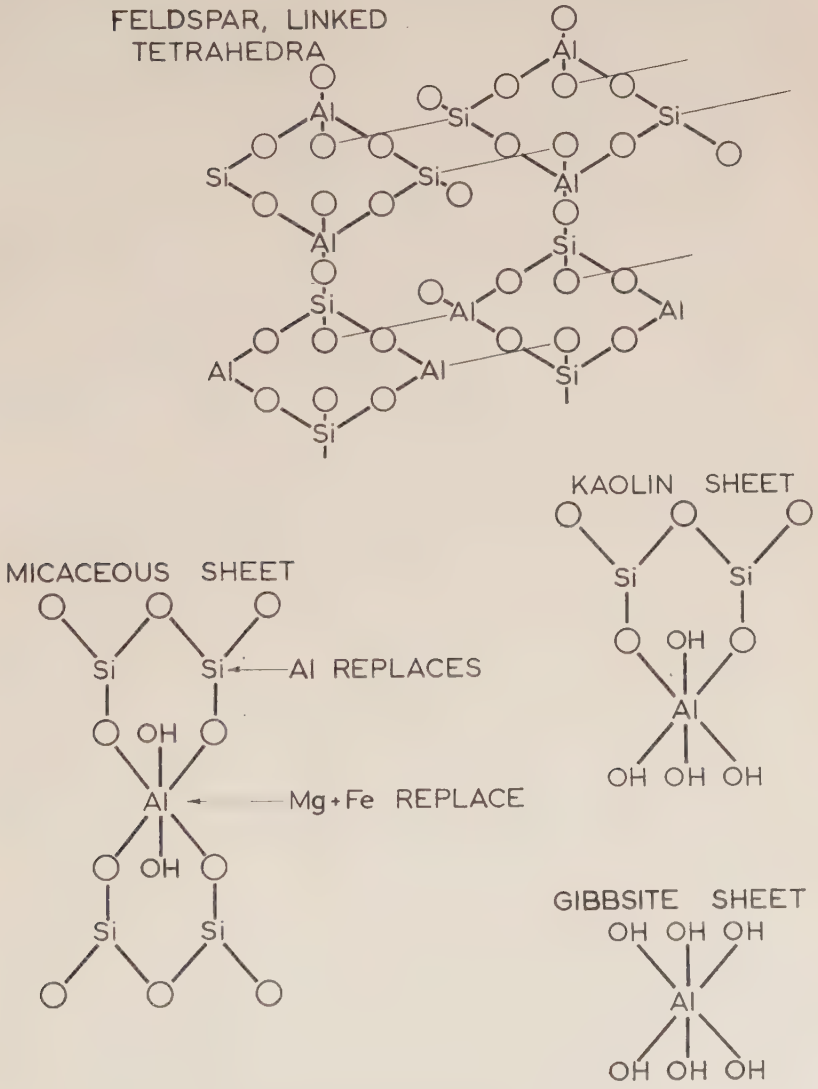


FIG. 8—Linkages in layer silicates, gibbsite and feldspar

Because of the disposition of the hydroxyls in kaolinite, there is likelihood that one-third of the sites due to type 1 fractures will not be neutralised by extension to type 2 fractures as in gibbsite. One will therefore expect $Al^{+\frac{1}{2}}$ sites from type 1 and 3 fractures to accept OH and become $AlOH^{-\frac{1}{2}}$ sites. Combination of type 1 and 3 fractures thus give rise to $AlOSi^{-\frac{1}{2}}$ and $AlOH^{-\frac{1}{2}}$ sites which are likely to be adjacent. At higher pH both sites will be negatived giving a negative colloid. At low pH both sites may accept a proton and have resultant half-positive charge. Since the sites differ, it is likely that $AlOH^{-\frac{1}{2}}$ will accept a proton at a higher pH than $AlOSi^{-\frac{1}{2}}$. At some intermediate pH, therefore, there will be adjacent $AlOH.H_3O^{-\frac{1}{2}}$ and $AlOSi^{-\frac{1}{2}}$ sites producing a local isoelectric condition. It is probable (Schofield 1949) that both sites develop positive charge below pH 5. According to Schofield and Samson (1954) positive charges arising from broken bonds on edge faces of acid-washed kaolinite may, in the absence of salt, result in flocculation of the kaolinite due to attraction to residual negative charge on the body of the crystals and may also bind negative platelets such as montmorillonite. This type of bonding may be of considerable importance in determining soil structure.

Both broken bonds of type 4 and type 5 fractures may be reduced to neutrality by hydrolysis resulting in uncharged SiOH or AlOH. As these are unlikely to dissociate in the pH range of soils the sites are likely to be inactive.

In poorly-formed kaolinites and halloysites other sites may be possible. These will be mentioned later.

(3) In *micaceous* type structures type 1, 3, and 4 fractures as in kaolinite are possible with perhaps little likelihood of type 2 effects. Charges on broken bonds thus vary in sign with varying pH.

In illites, clay-vermiculites, montmorillonites and other hydrous micas, isomorphous substitution in octahedral positions results in considerable permanent negative charge. This could affect the nature of the charge resulting from a fracture similar to type 3 thus:

$$Mg - OSi - Mg \rightarrow Mg - OSi^{-\frac{1}{3}} + {}^{+\frac{1}{3}} - Mg \text{ (Type 6 fracture)}$$

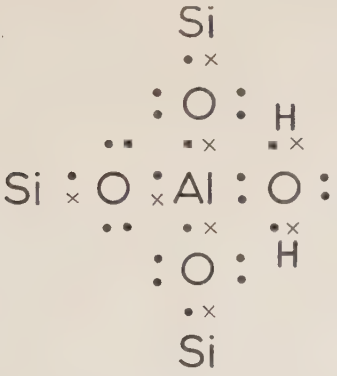
In micas also there is the possibility of substitution of Si by Al in tetrahedral positions. This type of substitution may still be present in hydrous micas such as illite, clay-vermiculite, and montmorillonite formed by degradation of mica. When present within the sheet structure it results in a permanent negative charge which is neutralised by acceptance of a proton when the site becomes accessible to water. At a broken edge, however, it results in a new type of fracture arising from a tetrahedral aluminium thus:

$$Al_{iv} - OH - Si \rightarrow Al_{iv}{}^{0} + {}^{0}OHSi \text{ (Type 7 fracture)}$$

Fractures of type 7 are common at broken edges of glass and feldspars and are discussed in the next section.

(4) In feldspar and glasses, type 4 and type 7 fractures are expected. Type 4 gives rise to uncharged SiOH sites. Type 7 fracture gives rise to an uncharged SiOH site and also to an uncharged tetrahedral Al site. The uncharged tetrahedral Al site is linked by oxygen to other tetrahedra thus:

The site is electrostatically neutral and stable but has octet instability as its outer shell contains 6 instead of 8 electrons. Furthermore, there is a hole in the uncompleted aluminium tetrahedron which will conveniently house an oxygen atom. In the presence of water or water vapour, therefore, it is likely that this site will be occupied by coordination with a water molecule thus:



If such a coordinated molecule were able to lose hydrogen by dissociation in the pH range 6 to 7·5, a particle of hydrated feldspar or glass would behave to cations like a synthetic cation-exchange resin and give rise to cation-retention effects similar to those observed with allophane. It is known that Al in the aluminate ion is in tetrahedral coordination but this is only in solutions at high pH. The pK of this dissociation is above 9 whereas the pK of the dissociation in the case of allophane would be below 7. Lowering of the pK to this value is thought to be made possible by internal linkages

within the solid to silicon; effects of this type in which increasing silicon lowers the pK values of alumino-silicates are discussed by Cook and Cutler (1953).

If this is so it seems likely that allophane B type clays (Fieldes, 1955) and palagonite (Fieldes and Williamson, 1955) are particles of hydrated feldspar or glass with many tetrahedral aluminium sites, some relatively inaccessible, exposed on all faces. At low pH there is little dissociation and in NaCl solutions chloride ions may compete with undissociated water molecules for the sites, giving chloride retention effects. With rising pH and dissociation an hydroxyl ion will be preferred to chloride so that chloride retention will diminish in a way which is not simply related to the uptake of cation.

Thus presence of tetrahedral Al could lead to mechanisms of cation and chloride retention which would fit the pattern of the results for allophane given here.

ANION RETENTION

The order of affinity for cations exhibited by clays and soils is reasonably well explained by the lyotropic series in terms of the charge on the cation and the size of the hydrated ion. These considerations do not explain affinity of clays for anions and it is generally thought that anion exchange or retention is brought about by mechanisms more specific than general electrostatic attraction. There must be very few soils indeed that do not contain some material similar to weathered feldspar or glass and it is of interest to consider how the proposed tetrahedral Al site would react to various anions.

In the first place an O is needed to complete the tetrahedron and the tetrahedral Al site therefore has a special affinity for oxygen. For this reason anions containing O will be preferred if their valence states are suitable. The likely stability of the adsorbed anion may be conveniently considered in terms of Pauling's (1945) electrostatic valence rules. Once an O atom has been accepted by the tetrahedral Al site the contribution of positive valence made to the oxygen by Al is $+\frac{3}{4}$. Since O has valence -2 an anion should form a stable linkage to the solid if the further contribution of valence to the O from the anion were $+5/4$. This will occur for anions containing oxygen tetrahedrally arranged around pentavalent elements such as phosphorus, vanadium, and arsenic. There is evidence that phosphates and vanadates exist in the soil pH range in tetrahedral coordination and fixation or strong retention of these anions by tetrahedral Al sites would be expected.

This concept can be extended and Table 2 shows the valence contribution to O by the central elements of a number of anions, and the resulting valence unbalance if such an anion were linked to a tetrahedral Al site. By this criterion maximum stability would be expected from conditions of least unbalance.

Table 2—Electrostatic Stability of Anions at Tetrahedral Al Site in Order of Increasing Stability

Anion	Valence Contribution to Oxygen (V)	Valence-Unbalance Site ($+5/4-V$)
Nitrate	$+5/3$	$-5/12$
Sulphate } Hydroxyl }	$+1$	$+\frac{1}{4}$
Acetate, etc.*	$+3/2$	$-1/4$
Carbonate	$+4/3$	$-1/12$
Molybdate Phosphate } Vanadate }	$+6/4$ to $+5/4$	$-\frac{1}{4}$ to 0
	$+5/4$	0

*In the case of acetate and other carboxyls, resonance is possible only between two C–O bonds which share three valence units of C, the fourth being directed towards the alkyl group.

Wells (1956) has reported the effect of various ions on molybdate retention by a soil from basalt. A measured amount of soil was shaken at near soil pH of 5·7 with a dilute solution of ammonion molybdate and the percentage Mo retention by the soil estimated from the loss of Mo from the solution. The test was repeated with additions of similar amounts of chloride, sulphate, phosphate, vanadate and at pH 8 and 9 with the following results:

	No Retention %	*pH*
Molybdate + chloride	64	5·9
Molybdate + sulphate	52	5·7
Molybdate alone	36	5·8
Molybdate + phosphate	0	5·7
Molybdate + vanadate	0	5·8
Molybdate + hydroxyl	18	8·0
Molybdate + hydroxyl	2	9·0

These results indicate a series of anion affinities in increasing order chloride, sulphate, molybdate, phosphate, and vanadate which agree well with values of Table 2. They also suggest sensitivity to variation of hydroxyl ion concentration. The latter effect may be quite complex as hydroxyl may affect the nature of the site and the configuration of ions in solution, and may also compete for the site.

A rather significant fact is the comparative stability a carbonate ion would have at a tetrahedral aluminium site (Table 2). It would suggest the way in which carbonate ion activity affects equilibria concerning phosphate and this effect needs consideration in work on phosphate availability.

Practical Considerations

Although existence of sites of specific activity on the surface of mineral colloids must be regarded as hypothetical at this stage, the above results indicate much in agreement with possible mechanisms of ion retention related to tetrahedral aluminium sites.

The following additional practical points may be mentioned:

(1) Montmorillonites are known to contain tetrahedral Al and the montmorillonite of Fig. 6 has been shown to have considerable variable negative charge above pH 6. This is true also for the Taita soil-clay and the Rothamsted subsoil, but these are likely to contain weathered feldspar.

(2) The existence of tetrahedral Al linked with tetrahedral Si in artificial aluminosilicates is accepted in the literature on cracking catalysts (Thomas 1949). The tetrahedral Al site is considered to be the active site in cracking and it may affect breakdown of organic matter adversely if too abundant. Cook and Cutler (1953) have also considered exchange mechanisms involving 4 — and 6 — coordinated Al in relation to the thermodynamics of base adsorption by clays.

(3) New Zealand brown granular clays from andesite have exceptionally strong phosphate and molybdate fixing power. Molybdate in particular is at deficiency level in sweet vernal grown on these soils (Wells, 1956) and pastures do not respond to applied soluble molybdate. The high free iron oxide content of the soils may well be a factor here but certainly the soils contain weathered feldspars. Tetrahedral Fe, which can occur in certain crystalline forms of hydrous iron oxide and may occur randomly in freshly precipitated hydroxide, should react like tetrahedral Al and may have even stronger affinities for anions.

(4) There is evidence of considerable phosphate and molybdate fixation in New Zealand yellow-brown earths at the stage when active weathering of feldspars is occurring.

(5) Poorly formed kaolins have high phosphate-fixing power. Where they are formed by resilication or from material derived from andesites or glasses, residual sites of tetrahedral Al are likely.

(6) When allophanes and palagonites are heated they lose considerable water at 105°C but rehydrate readily in humid air and the process can be repeated (P. G. Harris, pers. comm.). This may be understood since the sites will coordinate water molecules but not hold water or hydroxyl strongly at high temperatures where ionisation is limited by the dry state of the material.

(7) Clay minerals separated from New Zealand soils however weathered, or however siliceous, invariably have cation-exchange capacity measured at pH 7 of 10 me. per 100 g or greater. The presence of weathered feldspar or similar material may account for this.

(8) Lines of feldspars have frequently been noted in X-ray diffraction patterns of soil-clays. Hitherto they have been regarded as residues of inactive primary minerals but this view may need to change.

CONCLUSION

Allophane-type soil-clays have distinctive properties. In particular, they develop much negative charge in the range pH 6 to 7·5, and are strongly buffering in this range. The effect appears to be related to Al but is unlike

that of octahedral Al in known crystalline minerals. It seems necessary to assume that the Al in allophane is in tetrahedral form.

Broken bonds from tetrahedral aluminium sites would have special affinities for anions containing oxygen, and the order of affinities suggested by such a site agrees with those found in practice. The tetrahedral site also provides a satisfactory explanation of the mechanisms of development of variable negative charge by montmorillonite and palagonite.

Many soils contain weathered feldspar or similar material and the mechanisms suggested if valid may have wide application.

Titration curves or comparison of apparent cation exchange capacity at pH 7·5 and pH 5 in buffered acetate solutions would appear to provide ready laboratory means for evaluating amounts of conventional permanent negative charge and those of sites causing variable negative charge with selective attraction for anions.

Acknowledgments

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SOIL FROST ACTION IN SNOW-TUSSOCK GRASSLAND

By M. W. GRADWELL, Soil Bureau, Department of Scientific and Industrial Research, Wellington

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Summary

Observations have been made in the region of Fox Peak, South Canterbury, of the distribution of frost action in an area of snow-tussock grassland. Ice needles were found only on bare loamy soil, which occurred mainly where erosion terraces had locally replaced the tussocks. Growth of ice needles was prevented by a cover of tussock litter from ½ in. to 2 in. thick, according to the severity of the frost. The same protection was afforded the soil by 2 in. to 3 in. of scree overlying it. The ice needles on bare soil usually diminished in size towards the margins of adjoining tussocks, but, even at a distance of several inches, the larger needles could, by moving soil downhill, undermine a tussock and sometimes excavate and expose its roots. In the last stages of undermining, frost is probably a less important mechanism. In the soil of the cave underneath the undermined topsoil, which survives by virtue of the binding action of the tussock above, ice needles are less common than in the open.

Measurements of the diurnal fluctuations of soil temperature at a depth of 1 in. helped to explain these observations. Higher minimum temperatures and smaller overnight losses of heat were found under tussock litter than under an equal thickness of fine scree. A similar reduction of overnight losses of heat from the soil was observed where undermined tussocks overhung its surface.

INTRODUCTION

Previous observations on the forms and distribution of soil ice in a fescue-tussock grassland at Molesworth Station, Marlborough (Gradwell 1954, 1955) showed that serious disturbance of the soil by ice needles was confined to areas between the tussocks where the ground surface was bare of plants. Nowhere under a continuous plant cover more than a few inches in diameter was there seen sufficient ice to exert significant mechanical action on the soil. Large ice crystals grew more frequently on sunny aspects and ill-drained sites and were not found on soil which had been reduced by erosion to a texture too coarse to retain appreciable moisture. In general the distribution of ice crystals followed closely the local patterns of vegetation, erosion, and topography. It therefore seems that in a tussock grassland where the density of the ground-stratum plants between the tussocks and the incidence of erosion differ from those at Molesworth, the mode of action of soil frost could also be different. Such grassland is found in the high-altitude snow-tussock country of the South Island. This important vegetation unit covers about 5,000,000 acres and is in an advanced state of deterioration. It is thought possible that the element of climate that has the most profound effect on the stability of the soil in this grassland is frost (Tussock Grassland Research Committee, 1954).

Soil frost in snow-tussock grassland was studied at three areas in the catchments of the Opuha River, in inland South Canterbury. These were (1) an

area at about 3,600 ft near the Fox Peak ski hut and overlooking the Northern Opuha stream; (2) a higher area on the same face, around a saddle at 4,700 ft; (3) an area at about 3,700 ft near Bray's hut, overlooking the Southern Opuha stream and several miles from the first two sites.

DESCRIPTION OF AREAS STUDIED

Snowgrass tussocks (*Danthonia* spp.) were the dominant plants at all the study areas. The situation found on many sites at Molesworth, (Gradwell, 1954) where the soil between the tussocks of a continuous stand was bare of plants and open to frost action, was hardly represented here. The ground between tussocks was covered by low grasses or other plants or blanketed by a litter of snowgrass leaves. Bare soil was found mainly where erosion had locally destroyed the tussocks forming terraces of subsoil from a few feet to several yards wide. Where these terraces were small their surfaces were usually more gently sloping than were the tussock slopes around them and in all cases they appeared to be extending uphill by undermining the tussocks in that direction. The coherence of the topsoil between the tussocks, with its binding mass of roots, made this undermining action take place mainly in the subsoil. Thus the tussocks at the uphill margins of the terraces commonly stood at the top of steep banks a foot or two high or even formed cornices overhanging the bared subsoil underneath to a distance of several inches.

All the areas studied were on aspects which received the sun's rays directly at some time of the day during September, when the observations were made.

Fox Peak lower area (3,600 ft): Here the general slope was about 10° in a SW direction. Most of the area had a dense cover of small snowgrass tussocks with the intervening ground covered with low grasses, flatweeds, or tussock litter. Two large, steep erosion scars here had formed scree slopes on their lower reaches and were undermining the tussocks at their upper margins.

Fox Peak upper area (4,700 ft): Here a slope of about 5° on a southerly face was well covered with snowgrass tussocks and a ground stratum of small grasses. Erosion was confined to terraces a few feet in diameter. Some of these had a few inches of fine scree about their lower margins but in the main they consisted of bare subsoil.

Nearby, on a northerly slope of 5 to 10°, the surviving tussocks were several feet apart and served mainly to support the downhill margins of the numerous small terraces. Litter and small plants provided some protection but the soil was largely bare and covered to a considerable extent with a shallow layer of fine stones.

Bray's Hut area (3,700 ft): The area studied here was on W and NW slopes of about 10°. Here snowgrass tussocks with a ground stratum of low grasses were interspersed with fescue tussock (*Festuca novae-zelandiae*) and *Celmisia* sp. Many small subsoil terraces formed by erosion were present and these appeared to be extending rapidly and in some places working round behind the uphill tussocks. Some of these terraces were covered by snowgrass litter but others showed bare subsoil.

PROGRAMME OF OBSERVATIONS

Observations on soil frost were carried out in September of each of the four years 1955–8. In this month the snow cover of winter has usually just melted back from these areas and frost action in the soil is at its maximum. Observations were made at Fox Peak on the mornings of 17 and 18 September 1955. On these days the frosts were rather mild but the growth of ice crystals was not limited by soil moisture, as heavy rain had fallen on the preceding day. Observations were made at Bray's Hut on the five mornings 17–21 September 1956. Hard frosts occurred on the first two of these days and milder frosts on 19 and 21 September. Rather mild frosts were seen at Fox Peak on 17 and 19 September 1957. Severer frosts were observed there on 10 and 11 September 1958 and milder ones on 12–14 September.

In addition to visual observation of ice needles in the soil some measurements of soil temperature were made which, it was thought, might throw light on the microclimatic conditions responsible for differential occurrences of soil ice. The first series of measurements was made underneath the cornices at the upper margin of an erosion scar at the lower Fox Peak area. From 20 to 25 March 1959 readings were taken on mercury-in-glass thermometers placed at a depth of 1 inch in the soil. At this time of the year frosts did not occur but the elevation of the sun was similar to that in September, when observations of soil ice were made about the same cornices. Measurements were made at four sites and at each of them four thermometers were inserted in a line running at right angles to the edge of the cornices, so that the first thermometer was well under the overhang and the fourth out in the open. The arrangement at one of these sites is seen in Fig. 1, which is a downslope section through a cornice formed by undermining of the tussocks. Point a in Fig. 1 is vertically below the edge of the overhang and the positions of thermometers T1, T2, T3, and T4 are given (in distances from point a) as — 6 in., — 2 in., + 1½ in., and + 4½ in. (see Fig. 1). Distances under the overhang are taken as negative and distances out into the open as positive. Mercury-in-glass thermometers were used for this work, bent at right angles in accordance with the standard pattern of the N.Z. Meteorological Service. Readings were taken at intervals of 20 minutes around the times of minimum and maximum soil temperatures, which usually occurred between 6 a.m.–7 a.m. and 4.30p.m.–5.30 p.m. respectively. Less frequent readings were taken during the day. Thus accurate figures for daily minimum and maximum temperatures were obtained and the daily range of temperatures at each point computed.

Further readings of soil temperature were undertaken at the Soil Bureau Experiment Station, Taita, to compare the insulating properties of materials commonly found on the soil surface in snow tussock grassland. Four mercury-in-steel continuously recording thermographs (Negretti and Zambra type) were buried at a depth of 1 in. in a uniform and nearly horizontal plot of bare soil. Over one thermometer was placed a layer of snowgrass leaves 2 in. thick; over another a layer ½ in. thick; over a third a layer of fine stones 2 in. thick. The stones were mostly ½ in. to 1½ in. in diameter and light brown in colour. The soil over the fourth thermometer was left bare.

Occasionally the treatments were rotated, i.e. each surface cover was shifted to overlie the next thermometer, until readings for each treatment had been obtained from every thermometer.

RESULTS

Field Observations on Soil Ice

Of the forms of soil ice previously seen in tussock grassland at Molesworth (Gradwell, 1954) only needle ice and fine granular ice appeared to be common in the Fox Peak and Bray's Hut areas. It was noted that in nearly all situations ice needles melted during the day following their formation. Thus, although ice needles on bare ground reached 1 in. to 1½ in. in length during the severer frosts, tiers of needles as seen at Molesworth could not form. Multiple layers of ice crystals were observed at one place only on Fox Peak – under a bank where a continuous trickle of ground water fed the growing ice masses.

As at Molesworth, tussock cover was completely effective in preventing the formation of needle ice in the soil under the tussocks themselves. The same applied to ground-stratum grasses. Although the soil under these might be frozen, substantial ice crystals, capable of disrupting the soil, were never seen under a continuous cover of grasses, even where the grasses were very short. Needle ice developed fully on areas of bare loamy soil open to the sky: in such places the size of the needles seemed to be limited only by soil moisture and the intensity of the frost. A layer of surface soil, commonly from $^{1}/_{10}$ in. to ¼ in. in thickness, was raised on top of the ice needles, and, on sloping ground, this provided a mechanism for the downhill movement of soil and small stones.

The observations focussed mainly on situations intermediate between complete plant cover and bare loamy soil – i.e., on the effects of thin covers of snowgrass litter or small stones and on the occurrence of ice crystals about the margins of tussocks where these were bounded by bare soil. This occurred mainly with tussocks on the edge of erosion terraces and subject to undermining from below or to overrunning by debris from above.

EFFECT OF TUSSOCK LITTER ON THE FORMATION OF SOIL ICE

During the milder frosts, when ice needles on bare soils grew to lengths of $^{1}/_{3}$ in. to $^{2}/_{3}$ in., no needles were found on moist loamy soil under tussock litters ½ in. thick. Sometimes the soil underneath the litters was unfrozen and sometimes frozen to depths of one inch or more, but in the latter case the largest ice crystals in it were ice granules $^{1}/_{10}$ in. in diameter. The litters themselves were usually frozen crisp and dusted with fine crystalline hoar frost. In these milder frosts the same protection from the formation of needle ice was afforded to the soil by a complete cover of flatweeds. Quite small openings in the cover of litter or flatweeds were the sites of ice-needle formation – needles $^{1}/_{3}$ in. long were observed on patches of bare soil as little as 2 in. in diameter. In the milder frosts even litters thinner than ½ in. reduced the size of the ice needles underneath them to less than the size of needles found in bare soil nearby, providing that the litters were sufficiently continuous to render the ground underneath them invisible. However, litters

through which about half of the soil surface could be seen were underlain by ice needles as large as those in the open.

In a severer frost at Bray's hut, which produced ice needles more than 1 in. long in the open, litters ½ in. thick suppressed the growth of needles underneath them in some places only: at other points needles ½ in. long grew under such litters. Litters 2 in. thick suppressed the formation of ice needles everywhere. In another severe frost at the higher area on Fox Peak, needles grew an inch long in the open, but litters 1 in. to 2 in. thick suppressed them.

EFFECT OF FINE STONES ON THE FORMATION OF ICE IN UNDERLYING SOIL

Thin layers of small stones lying on moist soil were lifted off the surface by ice needles. Thicker layers of stones, however, provided sufficient thermal insulation of the soil underneath to suppress the growth of ice needles. On one occasion at Bray's hut when ice needles grew 1 in. long in the open no needles were found under 2 in. of small stones; on another day needles ½ in. long were present under 2 in. of stones but no freezing occurred under 3 in. of stones. At the higher area on Fox Peak also, ice needles ½ in. long were found under layers of small stones up to 2 in. thick. On the larger erosion scars, ice needles were widespread on the bare subsoil near the uphill and side margins of the scars, but did not appear under the thick masses of scree which covered their central and lower areas.

In some places a covering of small detached lumps of soil lying on the ground surface, and possibly residual from previous frosts, was seen to behave in a frost in much the same way as a layer of surface stones. The detached soil particles were apparently in poor capillary contact with the ground surface and so froze hard without drawing from below the moisture needed to form ice crystals. Where needle ice formed it grew from the moist ground surface below: the dry, frozen, soil crumbs were raised intact on top of the needles. A layer of such dry detached crumbs or flakes of soil apparently had a considerable insulating power. On one terrace only small needles or granules were found under the crumbs whereas on an adjoining soil surface with no loose crumbs ice needles 1 in. long were present.

OCCURRENCE OF ICE NEEDLES NEAR THE DOWNHILL MARGINS OF TUSSOCKS

In general, ice needles on bare soil became smaller towards the margins of Tussocks. Bare soil on the downhill side of tussocks usually took the form of an eroding terrace whose slope increased towards the tussock. Such a formation is illustrated in Fig. 2. In this figure the ice needles are shown as being shorter on the steeper portions of the slope, as was often observed. In some places the needles disappeared a short way up the slope; in others they continued in reduced size right up to the margin of the tussock. The closeness with which they approached the tussock seemed to be influenced by the severity of the frost and by the extent to which the tussock overhung the slope and sheltered the soil from above. At a few places small needles grew, in hard frosts, on almost vertical portions of the bank overhung by foliage to a distance of an inch. One tussock, overhanging steep slopes on every side except the uphill, had short ice needles growing on almost vertical portions of the

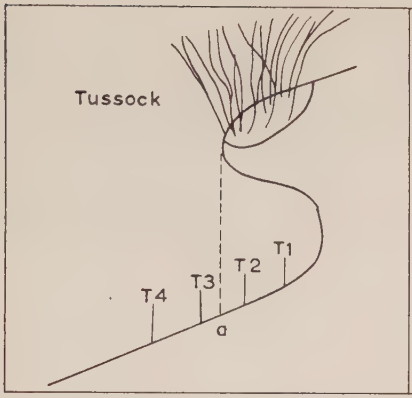

FIG. 1—Arrangement of thermometers at one site under a cornice.

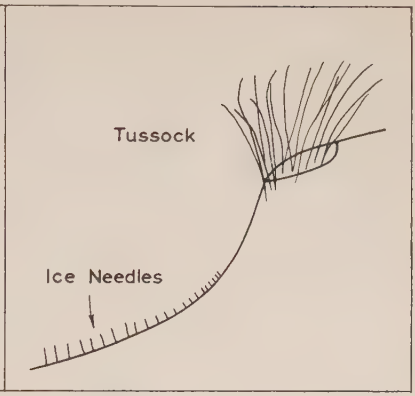

FIG. 2—Down-slope section through a tussock and the terrace downhill from it.

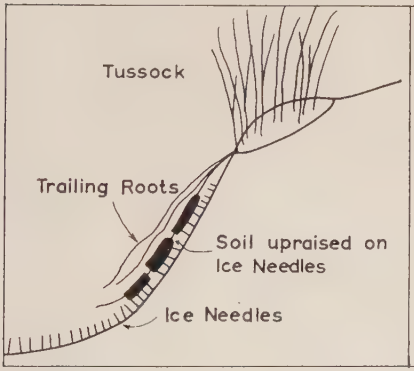

FIG. 3—Down-slope section showing ice needles growing behind trailing roots.

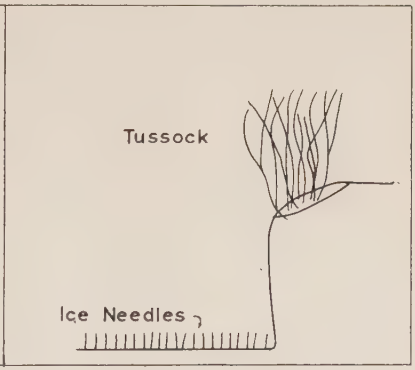

FIG. 4—Down-slope section through a tussock at the top of a vertical bank.

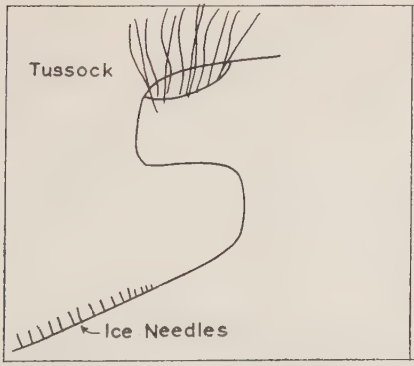

FIG. 5—Down-slope section through an undermined tussock.

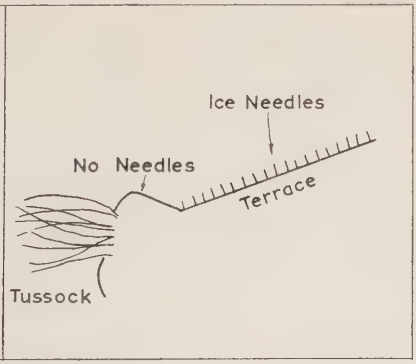

FIG. 6—Down-slope section through a tilted tussock.

slopes below it on three sides. In many cases erosion of soil from the steep bank immediately below the tussock had left exposed roots trailing down the slope. Ice needles grew behind these roots and in one place had lifted off the slope a layer of surface soil in which trailing roots were embedded (Fig. 3).

In many places erosion of a terrace had reduced it to a nearly horizontal surface at the foot of a vertical bank of earth several inches high. In such cases the ice needles on the horizontal surface usually extended, undiminished in size, right to the foot of the bank, below the tussock (Fig. 4) but although the bank itself was frozen hard usually only small granular ice crystals appeared in it. Occasionally, when the tussock did not overhang the bank, ice needles shorter than $\frac{1}{4}$ in. were found on the vertical surface and extending to within an inch of the plants at the top. When tussocks overhung the terraces below them to a distance of more than a few inches their shelter considerably reduced the formation of ice needles. The vertical faces showed no ice larger than grains and the needles on the horizontal terrace often did not extend to the foot of the bank. The thick leaves of *Celmisia spectabilis* were seen to be particularly effective in preventing the formation of needle-ice on banks over which they sprawled. The soil behind them was often found unfrozen even in severe frosts or, if frozen, contained only fine granules of ice.

Where erosion had gone a stage further and undermined the tussock it was usually noted that the ice needles on the floor of the terrace did not come much further into the cavern so formed than a point vertically below the overhanging edge of the tussock (Fig. 5). On three days on which observations were made ice needles were found outside but no more than 1 in. to 2 in. inside the caverns. On one such occasion recent rain had wetted the soil outside but the insides of the caverns were dry; on another day, however, the insides of the caverns were thoroughly wet but unfrozen. On a fourth occasion ice needles, $\frac{1}{4}$ in. long, were seen for the first time in a cavern, several inches under the overhang. This was after a period of fine weather which had dried up the surface soil in the open leaving, apparently, too little moisture for the formation of ice needles. The soil outside the cavern had frozen hard but with no visible ice crystals.

Occurrence of Ice Needles Near the Uphill Margins of Tussocks

Bare earth on the uphill side of a tussock usually took the form of a terrace stabilised by it. Sometimes this was covered by scree which was overrunning the tussock and in many instances the tussock was tilted downhill, raising the coherent clump of earth about its roots into a mound behind it. Where the soil was bare or covered with only a thin layer of stones, and the tussock untilted, ice needles were often seen to extend to an inch from the margin of the plant. In some cases smaller needles pressed right up to the roots. Where tilt of the tussock, or erosion of the terrace behind, had raised the clump of soil about the roots into a mound this was usually frozen hard but without ice crystals (Fig 6.) In one case only, needles $\frac{1}{8}$ in. to $\frac{1}{4}$ in. long were found on this mound, the needles on the terrace surface behind being $\frac{1}{2}$ in. long. The clump of earth thus free from ice-needle action contained the roots of the tussock, few roots being usually found on the terrace behind, where maximum formation of ice needles took place.

Readings of Soil Temperature

RESULTS FROM THERMOMETERS PLANTED BELOW CORNICES ON FOX PEAK

Overnight temperature drops (Maximum-Minimum) for four calm, clear nights in March are set out in Table 1. It can be seen that on each of the four sites studied the drop was smaller under the overhanging cornice than out in the open. The minimum temperatures attained on these days are set out in Table 2. On each site minimum temperatures were lower in the open than at the same depth in soil under the cornices. This relationship is illustrated in Fig. 7. Here minimum temperatures on 22 March are plotted against

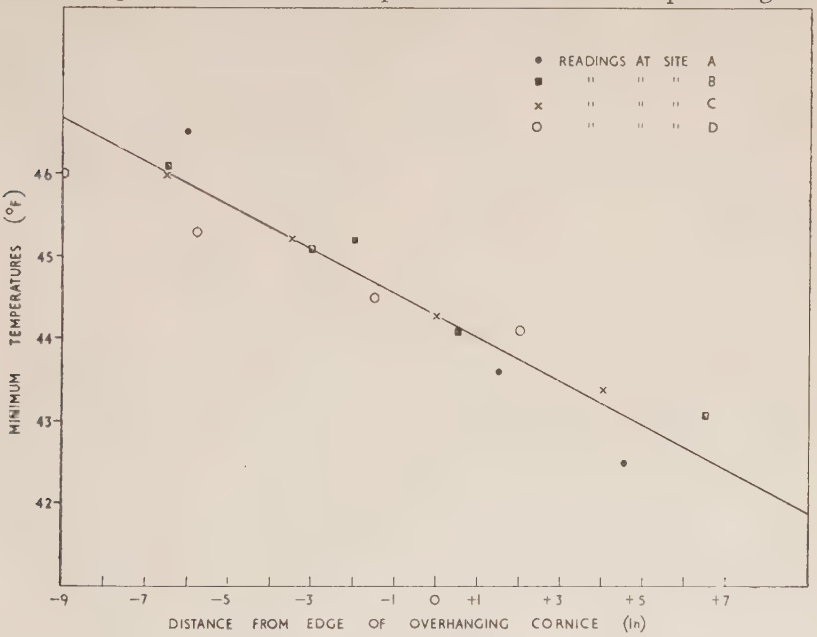

FIG. 7—Minimum temperatures at 1 in. depth in soil under cornices (22 Mar. 1959)

the distance of the point of measurement from the point directly under the edge of the overhanging cornice. Positive distances are away from the cornice into the open and negative distances are under the cornice.

RESULTS FROM THERMOMETERS PLANTED UNDER LAYERS OF LITTER OR SCREE

Overnight temperature drops (Maximum-Minimum) at a depth of 1 in. are set out in Table 3. The results are for four calm, clear nights (conditions similar to those prevailing during radiation frost). The minimum temperatures recorded on these mornings are set out in Table 4. It can be seen that surface covers, both of litter and of scree, substantially reduced the overnight loss of heat from the soil and raised the minimum temperatures. Two inches of snowgrass litter was the most effective cover for these purposes. Two inches of fine scree was nearer to one half inch of litter than to two inches of litter as regards its effect on the temperature of the soil underneath.

TABLE 1—Daily Ranges of Soil Temperature at 1 in. Depth Under Cornices (°F)

Position* (in.)	Site A				Site B				Site C				Site D			
	−6	−2	+1½	+4½	−6½	−3	+½	+6½	−6½	−3½	0	+4	−9	−6	−1½	+2
Date																
20–21 Mar.	3.6	4.8	6.5	7.3	5.3	6.2	7.2	7.2	5.3	5.7	6.3	6.9	4.8	5.2	6.7	6.3
21–22 Mar.	3.2	5.4	8.1	10.0	3.8	5.5	7.4	9.1	3.9	5.1	6.7	8.5	4.2	5.3	7.0	7.3
22–23 Mar.	3.1	4.6	6.5	7.6	5.1	6.2	6.9	7.1	5.1	5.6	6.2	7.0	4.7	5.3	6.7	6.2
23–24 Mar.	3.1	4.4	6.2	7.2	4.7	5.5	6.6	6.9	4.6	5.1	5.7	6.6	4.5	5.1	–	6.2

*Negative positions are distances under the cornice; positive ones distances out into the open in front of it (see Fig. 1).

TABLE 2—Minimum Soil Temperature at 1 in. Depth Under Cornices (°F)

Position (in.)	Site A				Site B				Site C				Site D			
	−6	−2	+1½	+4½	−6½	−3	+½	+6½	−6½	−3½	0	+4	−9	−6	−1½	+2
Date																
21 Mar	45.9	45.1	44.0	43.3	45.0	44.4	43.6	43.4	45.0	44.6	43.9	43.4	45.4	45.1	44.3	44.2
22 Mar	46.5	45.2	43.6	42.5	46.1	45.1	44.1	43.1	46.1	45.2	44.3	43.4	46.0	45.3	44.5	44.1
23 Mar	47.2	46.3	45.0	43.9	46.7	46.1	45.4	44.7	46.8	46.2	45.5	44.8	46.7	46.4	45.7	45.4
24 Mar	47.4	46.3	44.8	43.7	46.8	46.1	45.2	44.5	46.9	46.3	45.5	44.7	46.9	46.4	–	45.3

TABLE 3—Daily Ranges of Soil Temperature at 1 in. Depth Under Various Covers (°F)

Date (1959)	Under a Bare Soil Surface	Under ½ in. of Snowgrass Litter	Under 2 in. of Snowgrass Litter	Under 2 in of Gravel
18–19 June	25.5	15 5	6.9	12.5
24–25 June	22.4	14.0	5.5	10.9
2–3 July	21.8	14.2	7.5	11.4
21–22 July	24.9	16.3	6.3	13.8

TABLE 4—Minimum Soil Temperature at 1 in. Depth Under Various Covers (°F)

Date (1959)	Under a Bare Soil Surface	Under ½ in. of Snowgrass Litter	Under 2 in. of Snowgrass Litter	Under 2 in. of Gravel
19 June	35.5	39.4	42.6	40.0
25 June	32.0	34.7	38.4	35.2
3 July	32.8	35.7	39.9	36.4
22 July	32.9	37.1	42.1	37.9

DISCUSSION

In the areas of snow-tussock grassland studied the extent of disturbance of the soil by frost appeared to be determined by the extent of bare soil already present. A continuous ground stratum of plants provided complete protection and overhanging foliage also reduced the growth of ice needles in the soil underneath. The usual location of disruptive needle ice was on bare soil open to the sky. Even where erosion had removed the plant cover, a layer of tussock litter or of fine scree frequently protected the soil from frost action. The thickness of the layer necessary varied with the intensity of the frost. It seems clear that from ½ in. to 2 in. of tussock litter or 2 in. to 3 in. of fine scree is sufficient protection from many of the frosts encountered in the tussock grassland. Complete protection of the soil would probably be provided by plant or litter cover much less thick than the original closed cover of snowgrass tussocks.

Attack on the tussocks by frost appeared to take the form of remote undermining, accompanied by some exposure and breaking of roots. Large ice needles growing on bare ground, usually some inches from the tussocks, moved surface soil downhill, leaving the tussocks perched at the top of steep eroding slopes where they were liable to overturn. It seems probable, however, that in the final stages of tussock undermining, ice needles are less active than other eroding agencies, as the shelter formed by the overhanging tussock reduced their growth in the soil underneath.

There is little doubt that thermal insulation is the mechanism whereby tussock litter or scree prevents the formation of ice needles in underlying soil. On several occasions soil moisture under these covers seemed adequate to form ice crystals, yet none appeared. Covers of litter or scree 2 in. or less in thickness reduced the overnight heat loss from the soil from $^1/_3$ to $^2/_3$ of

the loss from adjoining bare ground. This insulating effect is equal to that recorded under the centres of fairly large tussocks (Gradwell, 1955). It appeared that a layer of litter was a more effective insulator than the same thickness of scree. A comparison was also made between the diurnal temperature ranges under these two covers and those recorded at different depths in an adjoining plot of bare earth. These results suggest that 1 in. of fine scree is equivalent to 1¼ in. to 1½ in. of soil overburden, and 1 in. of litter to 2 in. to 3 in. of soil overburden in terms of their ability to reduce the diurnal ranges of soil temperature.

Differences between the incidence of ice needles under overhanging cornices and in the open in front of them could in some cases be explained by differences in soil moisture between these two situations. On one occasion, however, ice needles formed outside but not inside, although the soil inside was perceptibly moist. On this occasion the lower overnight losses of heat and higher minimum temperatures noted under the overhang is considered to have been responsible for the failure of ice needles to appear there.

CONCLUSIONS

The areal extent of disturbance of a snow-tussock grassland by frost may be estimated from the extent of bare, finely-textured soil present in it.

A cover of plant material considerably thinner than the original vegetation would provide protection from frost.

Attack by frost on tussocks is largely by undermining, carried out by ice needles on adjoining areas of bare soil. Thus frost action contributes to erosion by expanding existing areas of bare soil into the surrounding grassland.

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THE PRODUCTION OF ^{35}S LABELLED SPORIDESMIN

By P. J. BROOK and R. E. F. MATTHEWS, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland.

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Summary

The isolation of ^{35}S labelled sporidesmin from *Pithomyces chartarum* grown on a defined medium containing inorganic sulphate is described. Toxin production by three strains of the fungus is estimated by the use of labelled material. In mature cultures sporidesmin is not confined to the spores but it is not established whether toxin is actually produced in other parts of the fungus. Growth and conidia formation are less sensitive to sulphur deficiency than is sporidesmin production. Sporidesmin is unstable in aqueous media in strong light, being converted to a mixture of at least two substances with solubilities differing from sporidesmin.

INTRODUCTION

The toxic principle in the fungus *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis (syn, *Sporidesmium bakeri* Syd.) was isolated by Synge and White (1959) and named sporidesmin. They give the empirical formula of the substance as: C_{19}. H_{21}. O_6. N_3. S_2. Cl. The availability of sporidesmin containing a radioactive tracer would be useful in a number of ways in further studies on the relation between the fungus and the facial eczema disease in sheep. From the formula given by Synge and White, ^{35}S appears to be a suitable tracer atom on the following grounds: (i) cost, (ii) half life (87 days), (iii) ease of measurement, (iv) ease with which the fungus can be grown on a medium containing the tracer, (v) ^{14}C is another choice for labelling the toxin but would enter a much wider range of compounds than would ^{35}S. Thus it should be substantially easier to isolate ^{35}S labelled material in a usable degree of purity with respect to the tracer.

This paper describes a method for the production and isolation of ^{35}S labelled sporidesmin and illustrates its use in the estimation of toxin from three strains of the fungus.

MATERIALS AND METHODS

Strains of P. chartarum

Strain C (Thornton and Ross, 1959) was used in all tests unless stated otherwise. Strains 73a and 57103 were provided by Miss J. M. Dingley from her collection.

Culture Methods

All chemicals for media were of laboratory reagent quality. For liquid cultures, the fungus was grown in 250 ml conical flasks containing 50 ml of nutrient solution. Growth and sporulation of *P. chartarum* depend on good

aeration. It was found that growing the fungus on filter papers impregnated with nutrient solution in petri dishes is an economical way of putting a large effective growing surface into a small volume of incubator space. For cultures on filter paper, Whatman's seed test $^{1}/_{64}$ in. papers of 9·0 cm diameter were used. Two papers were put in each petri dish and 6 ml of solution added. This volume was just sufficient to saturate the papers. Culture vessels were autoclaved, after addition of solution, at 15–17 lb per sq. in. for 20 minutes.

Inoculum was prepared from conidia harvested from colonies grown on potato dextrose agar. The conidia were washed by centrifuging in two changes of sterile distilled water and were then dispersed in a third lot of sterile distilled water. Spore concentration in the inoculum usually varied from 1×10^5 to $2{\cdot}5 \times 10^5$ per ml. Conical flasks received 1 ml of the suspension and filter papers 0·5 ml. The nutrient solutions tested for growth of the fungus are described in a later section. The pH of these nutrient solutions before autoclaving ranged between 6·6 and 7·0. Flasks were not shaken during growth of the fungus.

At harvesting the contents of the conical flasks were macerated in a Waring Blendor to give a uniform suspension. A spore count was made on a sample of the suspension with a blood counting cell. The fungus was then separated from nutrient solution by centrifuging at 1,500 r.p.m. for 15 minutes in the 550 ml buckets of an MSE Major centrifuge. Filter paper colonies were dried at approximately 25°C for several days in subdued light. A mixture of mycelium and conidia was then scraped away from the paper. This method of harvesting removed 75%–80% of the spores from the paper.

Isolation and Estimation of Sporidesmin

Sporidesmin was extracted by boiling the harvested material in absolute methanol for about three hours. When extracts contained a reasonably high toxin level the methanolic extract was used directly for chromatography. When concentration of the extract was necessary this was done in a rotary film evaporator under reduced pressure.

Chromatography was carried out using water (Synge and White, 1959) on Whatman No. 3 MM paper. For preparative runs the extract was placed as a band across the full width of the paper. For quantitative tests a series of spots containing graded amounts of the sample were chromatographed. To minimise streaking of the sporidesmin spot, narrow vertical strips were cut from the sheet of paper, leaving lanes 3·2 cm wide. Each sample of extract or sporidesmin standard was placed as a band across a lane in the paper, about 3 cm below the top of the vertical cuts in the sheet. In this way water was forced to move evenly through the band. Estimates of amounts of toxin were made from the size of the spots produced by the azide-iodine reagent as described by Rusell (1960). As a reference standard for spot size and R.F. value, pure sporidesmin suplied by Dr E. P. White was used. Sporidesmin was eluted from the paper in absolute methanol or diethylether.

RADIOACTIVITY MEASUREMENTS

Radioactive sulphur was supplied in the medium as inorganic sulphate (Amersham Code SJS1).

Radioactivity measurements were made on samples of liquid dried on to planchettes, using a Philips GM tube No. 18505. The counting efficiency for ^{35}S was about 5%. Distribution of radioactivity on filter paper strips was determined with an automatic scanning and recording apparatus (Atomic Accessories Inc., New York. Model RSC–5) modified to operate with a wide end window GM tube (G.E.C. type 2B2). This apparatus was used in conjunction with a Philips ratemeter (PW 4042).

THE SULPHUR REQUIREMENT OF *P. chartarum* FOR GROWTH, SPORULATION, AND SPORIDESMIN PRODUCTION

To find a suitable medium for adequate growth of *P. chartarum* in which the level of sulphur could be controlled, variations on a medium used by Steinberg (1941) were tested in order to study the sulphur nutrition of *Aspergillus niger*. One such experiment, in which the fungus was grown in conical flasks for 7 days at 25°C, is summarised in Table 1.

TABLE 1—Effect of Carbon Source and Nitrogen Source on Growth and Sporulation of *P. chartarum*

Medium No.*	I	II	III
Nitrogen source	NH_4NO_3 2·06 g	NH_4NO_3 2·06 g	Asparagine 2·5 g
Carbon source	Sucrose 50 g	Glucose 50 g	Glucose 100 g
Mycelial growth	Good†	Good	Good
Chlamydospore production	Numerous	Occasional	Occasional
Conidia production	Nil	$0·3 \times 10^6$ per flask	$8·1 \times 10^6$ per flask
Toxin production	—	—	+‡

*Amounts of material expressed as quantity per litre of solution. All three solutions also contained, per litre, $MgSO_4.7H_2O$, 0·25 g; K_2HPO_4, 0·35 g; $FeCl_3.6H_2O$, 0·51 mg; $ZnCl_2$ 0·3 mg; $CuCl_2.2H_2O$, 0·095 mg; $MnCl.4H_2O$, 0·12 mg; $(NH_4)_2MoO_4$, 0·02 mg.

†Growth comparable with that on media such as potato dextrose agar or potato carrot agar.

‡Detected by azide-iodine reaction on methanol extracts of spores plus hyphae. Quantitative estimates were not attempted in this experiment.

The effects of sulphur level on the fungus were tested using nutrient solution No. III of Table 1. Various amounts of magnesium sulphate were added, the magnesium content being kept constant by appropriate additions of magnesium chloride (except for the highest level of sulphur noted in Table 2). The results of one such test are summarised in Table 2 and Fig. 1.

At 8·7 μg/ml of sulphur, conidia production was still substantial but the content of toxin in dried spores plus mycelium had dropped below a detectable level. Thus sporidesmin production appears to be more affected by low

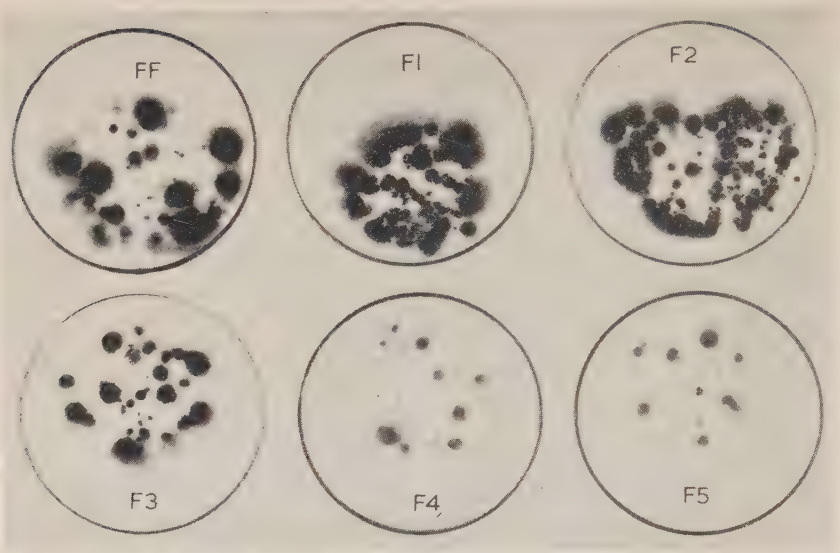

FIG. 1—Growth of *P. chartarum*, strain C, after 16 days at 25·5°C on filter papers with nutrient solution containing various levels of sulphur. The dark patches are where conidia have been formed. Nutrient solutions contained sulphur as follows (μg/ml): FF, 69·4; F1, 34·8; F2, 17·4; F3, 8·7; F4, 4·3; F5, 2·2.

levels of sulphur than is growth and sporulation of the fungus. It is unlikely that the increasing amounts of chlorine in the nutrient solution, which accompanied the decreasing sulphur levels, were important in reducing toxin production, since the largest increase in chlorine level in the media listed in Table 2 did not correspond to any significant fall in amount of toxin produced.

TABLE 2—Effect of Level of Sulphur in Nutrient Solution on Toxin Production by *P. chartarum* Strain C

Concentration of Sulphur in Nutrient Solution (μg/ml)	Concentration of Chlorine in Nutrient Solution (μg/ml)	Concentration of Sporidesmin (as a percentage by weight in dried spores plus mycelium)
69.4	0.454	0.12%
34.8	0.454	0.12%
17.4	39.5	0.10%
8.7	58.5	<0.0025%*
4.3	67.9	<0.0025%
2.2	70.5	Doubtful trace

Fungus grown on filter papers at 25·5C for 16 days. In the highest sulphur level, both sulphur and magnesium were approximately double that for nutrient solution III of Table 1.

*Taking 1 μg sporidesmin as the lowest limit of detection by the azide-iodine test.

THE PRODUCTION OF ^{35}S LABELLED SPORIDESMIN

For the production of ^{35}S labelled sporidesmin, nutrient solution No. III of Table 1 was used except that the 0·25 g of $MgSO_4 . 7H_2O$ was replaced by 0·192 g $MgSO_4 . 7H_2O$ plus 0·048 g $MgCl_2 . 6H_2O$. This concentration of sulphur (25 μg/ml) was chosen as that giving approximately the maximum yield of toxin per unit weight of sulphur supplied. To illustrate the method for producing labelled toxin details of one experiment are given.

Eighteen mc of ^{35}S sulphate in 3 ml of water was added to 200 ml of nutrient solution. This gave an activity for the sulphur (as measured in our equipment) of $3·2 \times 10^5$ c.p.m./μg S. The medium was dispensed in 6 ml lots in petri dishes, with filter papers. After 10 days' growth at 24°C the cultures were dried for 3 days at 23°C and the spores plus mycelium harvested (air dry weight = 1·29 g). The methanol extract of this sample contained a total of $7·5 \times 10^7$ c.p.m. Approximately 30% of these ($2·2 \times 10^7$ c.p.m.) were isolated in the sporidesmin band after chromatography in water. The evidence that most of this radioactivity is in the toxin is given in the next section. The eluted material contained a total of 570 μg of toxin estimated by spot size with the azide-iodine reagent. Thus the yield of sporidesmin was 0·044% of the dry weight of spores plus mycelium with an activity of $3·9 \times 10^4$ c.p.m./μg of sporidesmin.

The filter papers on which the fungus had been cultured were extracted in methanol. This extract contained $1·5 \times 10^8$ c.p.m., of which about 12% ($1·8 \times 10^7$ c.p.m.) was in the toxin band. Thus about half the total toxin was in the dried filter papers, although only about one fifth of the spores remained adhering to them. This suggests that a substantial proportion of the toxin is not present in the spores themselves.

For routine production of ^{35}S toxin it would be simplest (at least for the C strain) to extract the dried filter papers and fungal colonies together. In the experiment detailed above, if the whole culture had been extracted together a total of about $(2·2 \times 10^7) + (1·8 \times 10^7) = 4 \times 10^7$ c.p.m. in toxin would have been obtained. Allowing for a counting efficiency of 5%, this is equivalent to a yield of about 360 μc of toxin from 13 mc of inorganic sulphate.

PURITY OF ^{35}S LABELLED SPORIDESMIN WITH RESPECT TO SULPHUR

When a methanol extract of a mixture of dried mycelium and spores of *P. chartarum* strain C that has been grown on a medium containing ^{35}S is chromatographed in water about 95% of the radioactivity moves as two bands (Fig. 2, strain C). The band moving near the solvent front contains about 75%–80% of the sulphur. The nature of this material has not been investigated. Our evidence that the radioactivity in the slower moving band is at least very largely in sporidesmin is as follows:

1. The band moves in the same position as authentic sporidesmin in the water solvent.

2. It gives a reaction with the azide-iodine reagent. The intensity of this reaction as measured by comparison of a series of spots of graded size with a series of authentic sporidesmin spots is close to that expected from the amount of sulphur present (estimated by radioactivity) and the known composition of sporidesmin.

3. A sample of the material was eluted in ether together with an appropriate paper blank. The optical density at 252 mμ was measured in a Beckman DU spectrophotometer and the concentration of sporidesmin estimated (optical density for 1 μg/ml of pure sporidesmin (without CCl_4) = 0·029) (E. P. White, pers. comm.) The radioactivity measured on the same sample gave an estimate of the amount of sulphur present. From these data the calculated percentage of sulphur in sporidesmin was 12·7%. The formula of Synge and White (1959) requires 13·2%.

4. Provided the conditions for stability of the toxin noted below are maintained, the radioactive material eluted in methanol runs as a single band on re-running in the water solvent. It also runs as a single band near the solvent front in both benzene/hexane (50%/50%) and methanol/water (50%/50%).

STABILITY OF SPORIDESMIN

Early chromatographic tests with ^{35}S labelled sporidesmin in this laboratory resulted in erratic and unpredictable losses of label from the toxin band. Such losses have been found to be due to the instability of the toxin under certain conditions. While it appears to be quite stable on the bench in solution in absolute methanol or in dried chromatograms, the toxin is unstable in water in strong light. If a water chromatogram of ^{35}S labelled toxin is run in sunlight, a substantial proportion of the material may be converted to a mixture of at least two substances which run very close to the solvent front. For this reason all chromatography (at least that using aqueous solvents) should be carried out under conditions of low light intensity. The products formed from sporidesmin are readily soluble in water but virtually insoluble in ether. On chromatography in the benzene/hexane (50%/50%) solvent the products remain at the origin while sporidesmin runs near the solvent front.

SPORIDESMIN PRODUCTION BY THREE STRAINS OF *P. chartarum*

Strain C was compared with strains 73a and 57103. Previous assay by the the azide-iodine test indicated that strain 73a produced more toxin than strain C, while 57103 contained no detectable toxin. The strains were grown on nutrient solution No. III of Table 1 with 25 μg sulphur/ml. Each strain was grown for 8 days at 24°C on filter paper with 6 ml of nutrient solution per petri dish. The cultures were then dried for 3 days at 24°C and spores plus mycelium harvested. This material was weighed, and extracted in 2·5 ml boiling methanol for 3 hours. The filter papers were extracted in 12 ml of boiling methanol for the same time. Radioactivity was determined on samples of the methanol extracts. Other samples were chromatographed in the water solvent. The toxin bands located by scanning the chromatograms for radioactivity were eluted in methanol and radioactivity in the bands estimated by counting on planchettes. Results are summarised in Tables 3 and 4 and Fig. 2.

TABLE 3—Estimation of Toxin in Dried Spores plus Mycelium for Three Strains of *P. chartarum*

Strain	A Weight of Air Dried Spores plus Mycelium	B Total c.p.m. in Methanol Extract	C Total c.p.m. in Toxin	Weight of Toxin Based on C†	Percentage Toxin by Weight in Spores plus Mycelium
C	28 mg*	4×10^6	7.5×10^5	19 μg	0.07%
73a	29 mg	6×10^6	1.3×10^6	30 μg	0.10%
57103	21 mg	4×10^6	2.0×10^4‡	<0.5 μg	$<0.0016\%$

*All figures are based on the yield from one petri dish containing 6 ml nutrient solution with ^{35}S sulphate containing 3.2×10^5 c.p.m./μgS.

†Assuming all activity recorded in column C was in toxin and that pure toxin has 13.2% sulphur.

‡This maximum figure is based on an estimate of the minimum area of toxin peak that could have been clearly observed above the background in a scan of the chromatogram at $\times$ 10, the instrumental amplification used for Fig. 2.

TABLE 4—Estimation of Toxin in the Filter Papers Used for Growth of the Three Strains of *P. chartarum* Noted in Table 3

Strain	Total c.p.m. in Extract	Total c.p.m. in Toxin	Weight of Toxin
C	1.9×10^7	6×10^5	14 μg
73a	1.3×10^7	1.2×10^6	28 μg
57103	2.0×10^7	$<2.0 \times 10^4$	<0.5 μg

Strain 73a contained more toxin than strain C, while strain 57103 had no detectable toxin band. When the chromatograms were scanned at a 10–30 times higher amplification than shown in Fig. 2, a low level of radioactivity was found along the full length of the chromatogram between the main peaks for all strains. This level was lowest for strain C, slightly higher for strain 73a and substantially higher for strain 57103. It is this general distribution of radioactivity along the paper (about 5% of the total for strain C) which places a limit on the minimum amount of toxin that can be detected in unfractionated methanol extracts.

DISCUSSION

From the chemical point of view the ^{35}S labelled sporidesmin preparations isolated by the simple procedure outlined above may well contain a substantial proportion of other methanol soluble compounds which do not contain sulphur and which have little absorption near 250 mμ. An increased degree of purity (along with unavoidable losses of material) could be obtained by applying the procedures which were developed for the isolation of crystalline toxin (Synge and White, 1959). However, with respect to sulphur, the data suggest that the sporidesmin is sufficiently free of

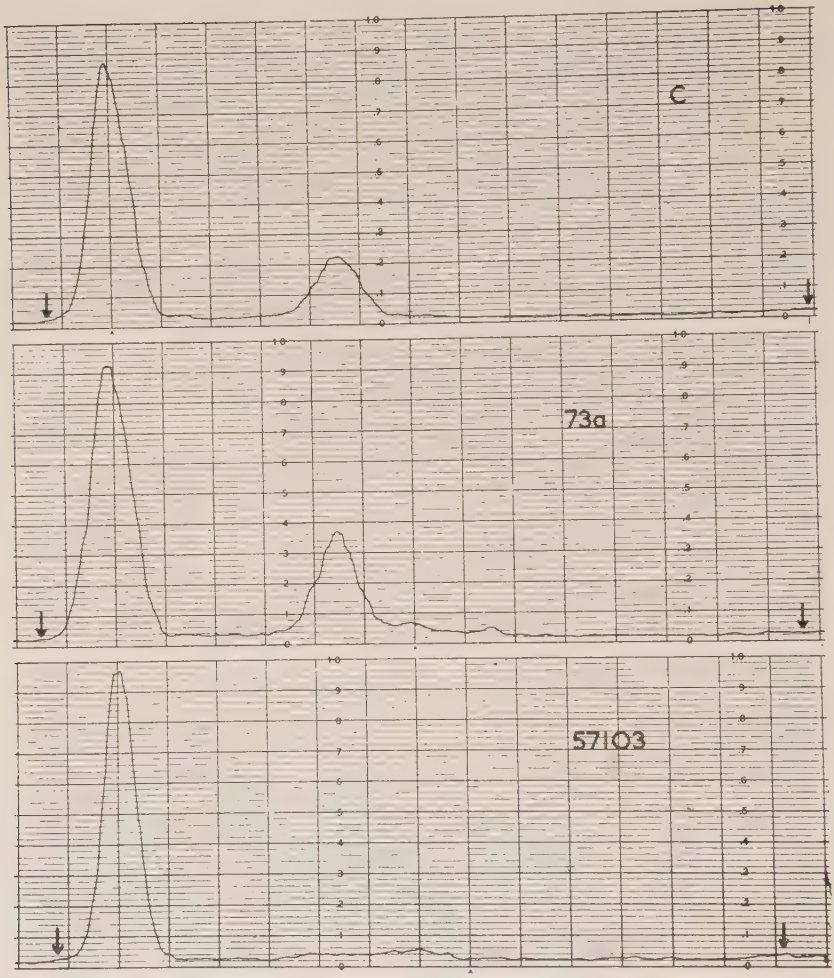

FIG. 2—Traces recording the distribution of radioactivity on water chromatograms of methanol extracts from spores and mycelium of strains C, 73a, and 57103 of *P. chartarum*. (Experiment of Tables 3 and 4.) Arrows indicate origin (right) and solvent front (left).

contamination to be useful for certain investigations. These could include: (i) the distribution of toxin within the fungus, (ii) the production of toxin by different strains of the organism, (iii) the testing of possible methods for coupling the toxin to an antigenic molecule, for example, some suitable protein, (iv) the exploratory tests on certain chemical properties of the molecule. For studies on the physiology and biochemistry of the toxin in the animal body a higher degree of purity may well be needed. The ^{35}S label would, however, be of little value if it turns out that the sulphur in sporidesmin is lost from the molecule at an early stage of its metabolism in the animal body.

The experiment summarised in Table 2 shows that, on the medium used, growth and conidia formation are less sensitive to sulphur deficiency than is sporidesmin production. This suggests that sporidesmin does not have any vital role in growth or sporulation of the fungus.

The conversion of sporidesmin to other substances which were noted in aqueous media in strong light may well take place under certain conditions in the field. The possible extent to which this can occur in pasture will be of particular interest if animal tests show that the products are not toxic.

Acknowledgments

The authors wish to thank Dr E. P. White, Mr N. T. Clare, and Mr G. R. Russell for most useful discussions; Miss J. M. Dingley for providing strains of the fungus, and Dr E. P. White for samples of sporidesmin. This work was supported in part by a grant from the Rockefeller Foundation.

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A GROWTH STUDY OF *Oxalis latifolia* H.B.K.

By D. I. JACKSON,* Canterbury Agricultural College, Lincoln, New Zealand.

(Received for publication, 20 June 1960)

Summary

A study was made on the seasonal growth and method of reproduction of *Oxalis latifolia* H.B.K. The stem and leaves of this plant are modified to form bulbs. A bulb consists of a central cone of stem tissue and two types of scales, termed membranous and true scales. The former scales may extend from the apex to form fully developed leaves and may thus be regarded as expanded leaf bases. In their axils may be produced inflorescences. The true scales cannot extend to form leaves and from their axils underground laterals may be produced which terminate in bulbils. Generally one series of each type of scale is produced in one season. At the base of the bulb is a thick tap root which contracts and pulls the bulb downward at the onset of winter.

Dormancy of bulbs and bulbils can be induced by inadequate moisture or nutrients, cold temperatures, and possibly any conditions unfavourable to growth. Dormancy can be more easily induced in bulbils than in bulbs.

Experiments with cold night temperatures and different day-lengths had no obvious effect on bulbil production or flowering during the season of treatment.

INTRODUCTION

The importance of species of *Oxalis* as weeds in New Zealand and the difficulty of controlling certain bulbous species is well known. Attempts to eradicate *Oxalis* of the latter type have met with only limited success: control by cultivation is effective only if relentlessly pursued, contact or translocatable sprays are only moderately successful and complete sterilisation of the soil, although effective, has many obvious drawbacks. Further, more extensive work on *Oxalis* control is warranted and, towards this end, a knowledge of the growth and reproduction is indispensable.

In New Zealand *Oxalis latifolia* H.B.K. and *O. pes-caprae* L. are probably the most troublesome species of *Oxalis*. The former grows during the summer and dies down in winter, while the reverse happens with the latter. The life-history of *O. pes-caprae* has been studied by Ducellier (1914) in Algeria, and Clarke (1934) in Australia. Observations reported below were made on the growth habit and form of *O. latifolia* (Fig. 1) at Lincoln, in the autumn of 1958 and for most of the growing season (November-April) 1958–59. A number of experiments were carried out in an effort to assess the effects of temperature and day-length on flowering and on formation and germination of bulbs and bulbils.

A GENERAL DESCRIPTION OF *Oxalis latifolia* WITH EMPHASIS ON GROWTH AND VEGETATIVE REPRODUCTION

Flowers of *O. latifolia*, and indeed most *Oxalis* species, are trimorphic, and for effective pollination and seed set, cross pollination between flowers of different stylar lengths is required (Salter, 1944). All flowers found growing

*Now on the staff of Fruit Research Division, Department of Scientific and Industrial Research, Havelock North.

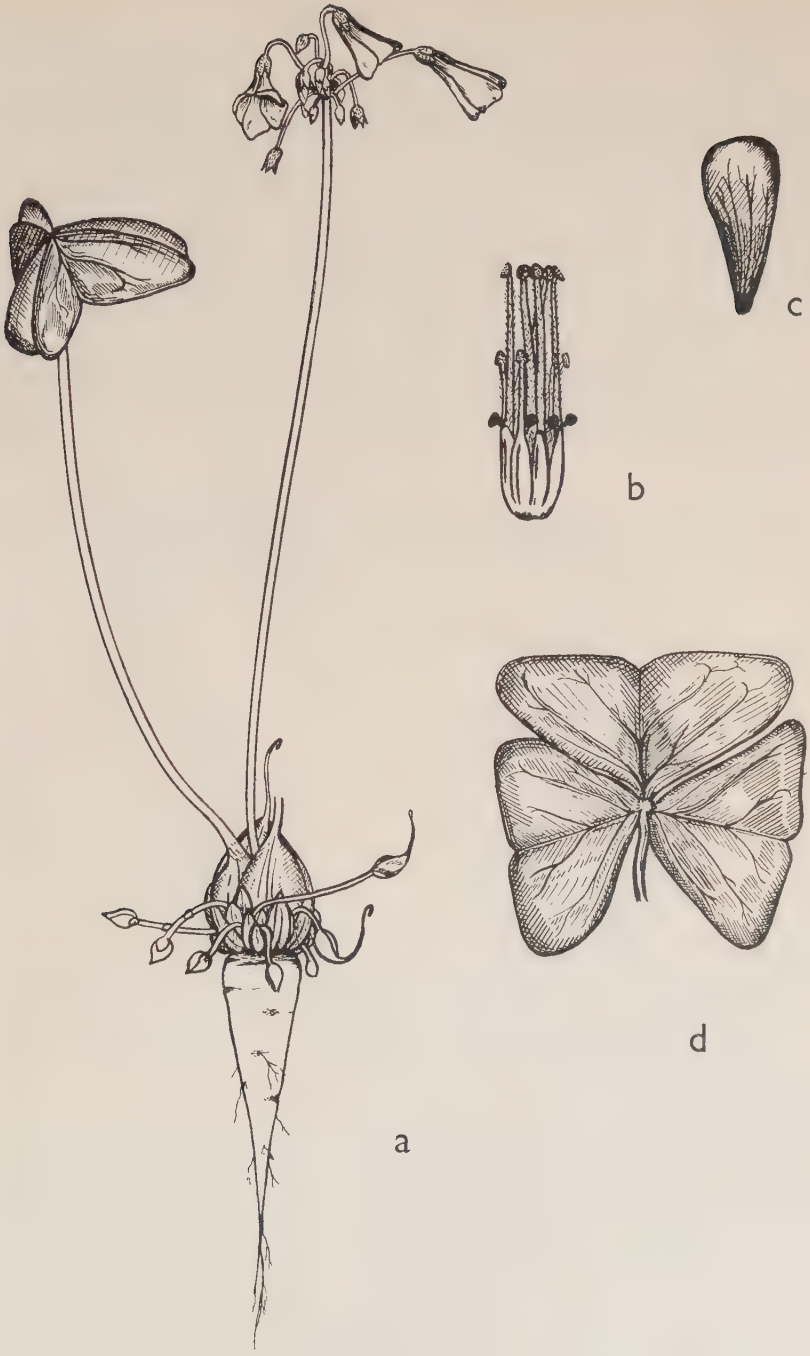

FIG. 1—Habit and form of *Oxalis latifolia:* (a) Entire plant, × 5/6; (b) Androecium, showing two whorls of stamens above, with stigmas below, protruding from within, × 5; (c) Petal, × 1·5; (d) Leaf, × 5/6.

at Lincoln and Christchurch were of the short stylar type due probably to the original introduction of plants to New Zealand as clones rather than seeds; lack of any other flower types accounts for failure to set seed, both here and elsewhere in New Zealand.

Bulbs are generally 2–3 cm long and the true stem of this plant is reduced to a cone of tissue in the centre of the bulb; it is from this cone or stem that the scales of the bulb originate. Scales (Figs 2, 3) are of two main types: (A) membranous scales, moderately thick at the centre and thinner towards the membranous hairy edges, colourless, bearing at the tip the rudiments of petiole and trifoliate leaf (which can extend to give fully developed leaves); these scales are closely appressed and one may extend completely around the bulb. (B) true scales, thick, fleshy, glabrous, brownish, somewhat triangular in cross section and relatively narrow, tapering to a pointed apex and apparently not capable of producing leaves.

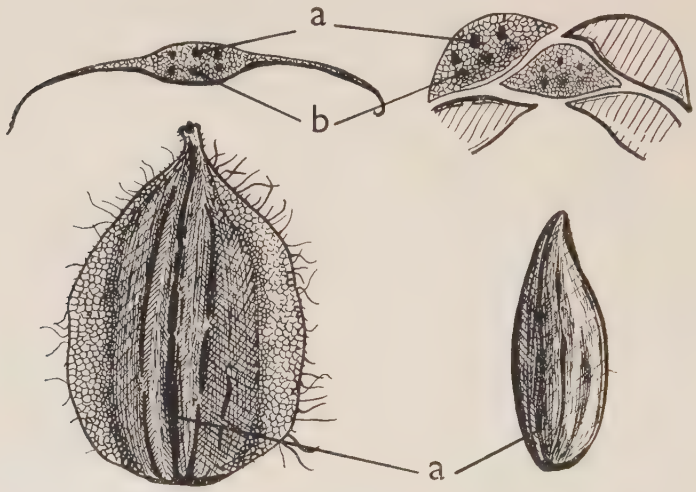

FIG. 2—The two types of scales produced by *O. latifolia:* Left: membranous scale, transverse section and entire scale – abaxial surface. Right: true scale, transverse section through group in natural position and entire scale – abaxial surface.
(a) Strands of pigment; (b) Vascular strands. × 4.

A third type which are seen on underground laterals and at the base of bulbils, are small, thin, of irregular shape and lack the ability of forming into leaves. From their axils further laterals may be produced. They are possibly undifferentiated true scales and will be referred to as scale leaves.

An orange granular pigment is contained in three strands grouped near the centre line and converging at the apex in each scale type. Beneath these strands, i.e. on the adaxial side, are very minute vascular strands which also traverse the length of the scales and converge at the apex, unlike the pigment however, these extend upwards along the petiole to the leaflets when these form.

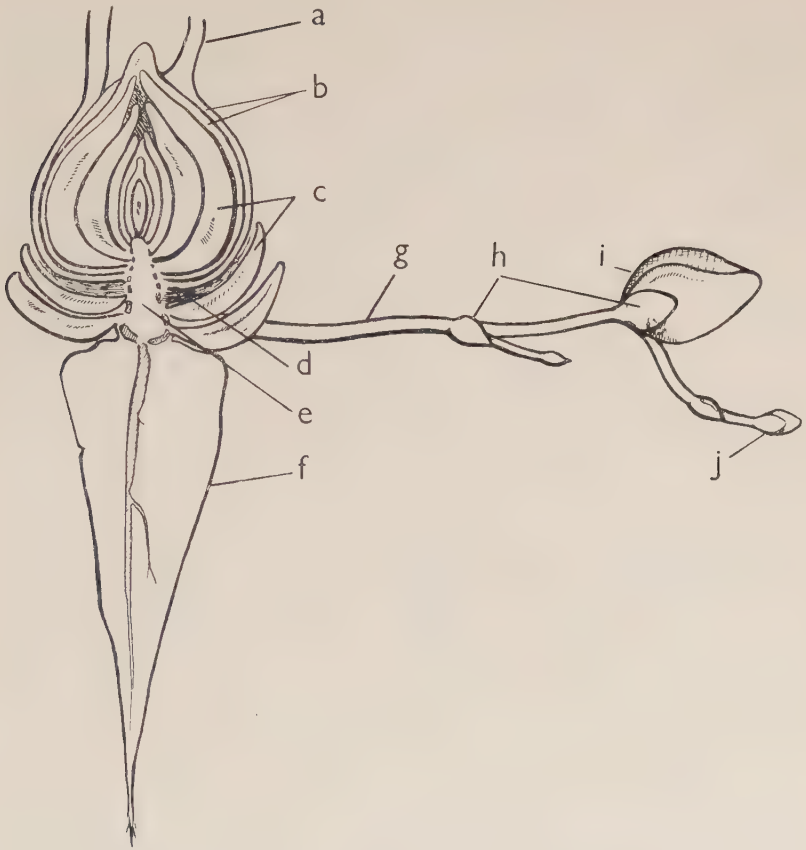

FIG. 3—Longitudinal section through bulb of *O. latifolia* showing position of stem and scale types in relation to other underground organs (drawn in January): (a) Base of petiole; (b) Membranous scales; (c) True scales; (d) Remains of outer membranous scales; (e) Stem tissue; (f) Tap root; (g) Underground lateral; (h) Scale leaves; (i) Bulbil (primary); (j) Bulbil (secondary). × 3.

From the base of a bulb, in the axils of true scales, underground laterals are produced. These laterals are white, fleshy and each may extend up to 4 cm before terminating in a bulbil. Occasionally, however, the laterals may branch to form more than one bulbil or the bulbil itself may produce additional laterals and bulbils. A bulbil attached by a lateral to a mother bulb is defined here as a primary bulbil, while if it produces bulbils of its own, when still attached to the parent, these further bulbils are termed secondary bulbils.

From the base of the cone of stem tissue adventitious roots arise. Generally one, occasionally more of these begins to thicken early in the season and eventually becomes dominant. This will be referred to as the tap root.

Bulbils, unless formed early in the season, seldom develop such a root until the following season. The tap root is sweet to the taste, translucent and brittle. Its contraction, generally at the onset of dormancy, serves to pull the bulb downwards through the soil. This contraction of the tap root is not large, since the fleshy part of the root rarely exceeds 6 cm in length and contraction may be halted by premature rotting of the root. Its nature has been discussed for *Oxalis incarnata* and *O. pes-caprae* by Thoday (1926), Thoday and Davey (1932) and Clarke (1934). Apparently demand for water by other organs of the plant, causes dehydration and collapse of certain cells of the root. Alternation of horizontal layers of collapsed and turgid cells gives the contracted root a concertina like appearance. At about the time dormancy is induced in *O. latifolia* bulbil production is prolific. It is probable that water and nutrients required for the process are extracted largely from the contracting root. The downward movement of the bulbs and lateral spread of bulbils effectively distribute the plant through the soil (Fig. 4).

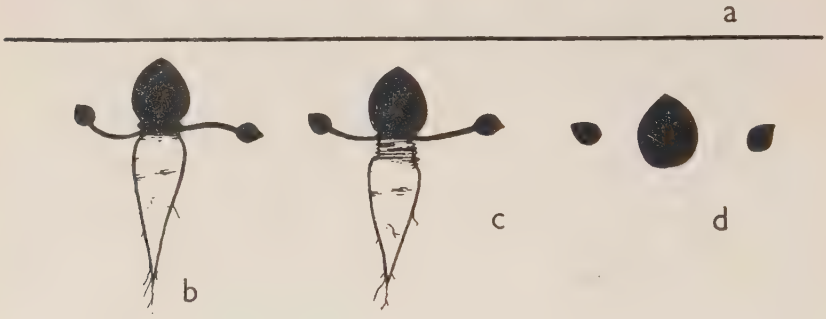

FIG. 4—Diagrammatic representation of movement of *O. latifolia* bulbs in soil: sequence of events (b–d) taking place at end of growing season (April–May): (a) Soil surface; (b) Bulb and bulbil before contraction; (c) Contraction taking place; (d) Final position of bulb and bulbil in the soil after decay of laterals and tap root. × ¾.

Bulbs are formed from bulbils in the following manner. Next to and within scale leaves at the base of a bulbil a series of membranous scales are formed which, if conditions for growth are good, extend to form leaves. Under less favourable conditions of light, moisture, temperature, and possibly nutrition, these membranous scales often do not extend to form leaves. Meristem at the apex dies, and they serve only as a protective cover for the developing true scales. Under such circumstances the bulbil seems to enter into a state of dormancy. Generally eight to ten membranous scales form before true scales begin to develop. Scales originate from the apical meristem in a spiral fashion, the apex becoming completely covered by enveloping scales. It appears from their mode of formation that all scales are undeveloped leaves; membranous scales can develop further to form leaves, true scales cannot.

In the first season a bulbil may produce several leaves, a few laterals with secondary bulbils, as many as 30–40 true scales inside the membranous scales and a series of adventitious roots. In the axils of membranous scales are generally to be seen inflorescence initials, but these, in the season 1958–59 (which was a dry one), seldom developed. After frosts have killed top growth in autumn the outer membranous scales die, becoming brown and solely protective. Further bulbils may form from the bulb, but as winter progresses bulbil production stops, and most become severed from their parents through decay of laterals, roots die and bulbs and bulbils assume a state of dormancy. Bulbs and bulbils are killed by temperatures below freezing point, and it is usual for no viable bulbs to be found in the top inch or two after winter at Lincoln. This probably explains why in Britain and parts of New Zealand where frosts penetrate to a greater depth this species of *Oxalis* is not a problem as a weed.

In spring, when soil temperatures reach about 60°F germination* of bulbs occurs. Last season's bulbils, having lost their attachment to parent bulbs, are now termed bulbs. The development of these newly formed bulbs is the same as that for last year's bulbs. Leaves appear from the centre of bulbs, and at the same time, from the axils of true scales a new series of laterals is produced, beginning at the more basal scales and extending upwards. By the end of the summer the number of bulbils formed can be as many as the number of true scales produced in the previous season. Ten to 14 leaves are commonly produced at the centre of the bulb from a new series of membranous scales. Within these again are produced additional true scales, generally slightly more in number than were produced in the bulbils. No more membranous scales are produced as a rule from the centre until next season. Each membranous scale has an inflorescence initial in its axil but many young inflorescences die before emerging from the bulb. Plants growing in a sunny situation generally produce more inflorescences than those growing in shade.

Some scales are transitional between the two types and it is not always a simple task to find where one type finishes and the other begins. When membranous scales are removed artificially and the bulb, with only the inner, true scales, replanted, a second set of membranous scales can sometimes be produced from the centre in the same season. In such a case, fully developed true scales never extend to form leaves, but no doubt production of these scales is forfeited for membranous scale production.

DORMANCY

Some Observations and Trials

Difficulty in inducing bulbs and bulbils to grow in a heated glasshouse during the winter, suggested that some dormancy phenomenon was involved. Bulbs taken from outdoors before leaves were killed by frosts in April, and placed in pots in the glasshouse, grew throughout the winter. Those brought indoors after frosts had occurred were difficult to germinate.

*The word germination in this case is used to indicate recommencement of leaf, flower, and lateral production after a period of dormancy.

On 16 July, in a trial to test the temperature effect on germination, three temperature treatments were applied to three different sets of bulbs. Each set consisted of twelve 3 in. pots, six containing three of last season's bulbils apiece and six with one bulb per pot. All were planted one inch below the surface. A potting mixture of two parts soil, two parts sand and one part peat was used. The first set was placed in a heated room with a temperature varying between 65° and 75°F, the second in a corner of a glasshouse where winter temperature varied from 35°–65°F, and the third set outdoors. Bulbs and bulbils of the last of the three sets were all killed by frosts.

After one month, one shoot had appeared from a bulbil of the first set, and a month later, another appeared. It was early October before any appreciable germination took place and both bulbs and bulbils sprouted at the same time. *Oxalis* kept under glass germinated in late October while shoots appeared above ground outdoors about 10 November. Another set of bulbs were stored at a temperature of 34°F for 9 months. These germinated quickly when brought out of storage in January. Soil temperatures were recorded when the first *Oxalis* appeared outdoors in November and were found to average about 65°F. Until a certain period of time has elapsed high temperatures alone seem incapable of inducing germination once dormancy has become established. If dormancy begins with the frosts of April-May and ends between September and October (the time when bulbs in the glasshouse and heated room germinated) then 4–6 months are needed to break dormancy.

The form of the dormancy phenomenon indicated above is as follows: all growth above ground dies and no further leaves or inflorescences are produced; growth below ground slows down bulbil production, although it may be temporarily stimulated by poor conditions, eventually ceases, roots die and presumably activity is confined to slow proliferation of scales at the stem apex.

Conditions Affecting Length of Dormancy Period

Warm temperatures in a heated room, moderate temperatures in a glasshouse or cool temperatures of soil outdoors had little effect on the length of the dormancy period. The effect of other growth factors on the length of dormancy was not investigated.

Ethylene chlorohydrin applied as recommended by Crocker (1948), 3½ months after the induction of dormancy, did not induce earlier growth in bulbs kept at 65°–75°F.

Factors Inducing Dormancy

Bulbs brought from outdoors and grown in a warm glasshouse during April and May 1958, produced many bulbils. These were comparatively small and consisted of 2–4 membranous scales, each of which had extended to form a leaf. As winter progressed, glasshouse temperatures became cooler, the amount of light less, and the supply of nutrients in the soil depleted by heavy earlier growth, a change in bulbil production was noted. Bulbils tended to gain in size due to formation within the membranous

scales of a series of true scales, leaf formation ceased and bulbils entered what was probably a dormant state. Bulbils formed at this later stage sent up no leaves and these also remained dormant.

To confirm this effect of nutritional status some bulbs in 5 in. pots which germinated under glass in September in a mixture of 50% peat and 50% sand, were moved outdoors in November. Thirty pots were watered with a nutrient solution containing nitrogen, phosphorus and potassium while another 60 were given water only. Top growth in the latter set was weak and chloritic and confined mainly to bulbs; bulbil production was prolific but few of these sent up leaves, and most remained dormant. Apparently under low nutrition, dormancy is induced. Before applying nutrients to the other set, it was noticed that many apparently dormant bulbs were present. A few of these sprouted after receiving nutrients; many still remained dormant. Bulbil production was not so prolific as in the low nutrient set but of these bulbils many sent up leaves. While this was not intended as an exhaustive trial to test the effect of nutrition on bulbil production, results did suggest that low nutrition may cause dormancy of bulbils. It was noticed that a bulbil can send up a few leaves, and then become dormant if conditions become unsuitable.

Plants growing outdoors in the dry season of 1958-59 had many leafless bulbils which were probably dormant. Heavy rains experienced in March did not cause many of these to form leaves, although temperatures were still quite high. Lack of moisture rather than lack of nutrients probably caused this dormancy.

Under unfavourable conditions bulbs tend to produce bulbils which quickly become dormant. How many days or weeks of poor conditions are needed to bring about this dormancy is not certain. The unfavourable conditions are poor nutrition, lack of moisture, cold temperatures and possibly, in the case of plants grown under glass during winter, low light intensity, with a corresponding low synthesis of carbohydrates. In all cases noted, except cold, it was bulbils and not bulbs in which dormancy was induced. Possibly severe drought or extremely poor nutrition would induce dormancy of bulbs. Whatever the cause of this dormancy and whenever it is induced reversal to ideal conditions will not break it unless a given period of time has elapsed.

Effect of Day Length and Night Temperatures on Flowering and Bulbil Production in *Oxalis latifolia*

Twenty 3½ in. pots were planted on 20 November 1958, with *O. latifolia* bulbs that were germinating, but whose leaves had not appeared above the soil surface. Ten were held until the following March under short days (S.D.) of 8 hours, while the other ten were held under the long days (L.D.) of summer for the same period. The only apparent difference between the two sets was that the L.D. plants showed slightly more vigour early in the season, while the S.D. plants were more vigorous later, due perhaps to the depletion of nutrient supplies earlier by the L.D. plants. Both sets flowered at the same time and gave a similar number of inflorescence

stalks; no differences in the number of bulbils or their dormancy was discovered. Initials of both inflorescences and laterals could be seen in the axils of scales on germinating bulbils before planting, and it may be, that if day length does have any effect, it is in the previous season. Although membranous scales were not apparent in April 1958 their initials may have been formed, and so also the initials of the inflorescences. The same bulbs would have to be grown for another season under similar day-length conditions to give a full picture. The two day lengths used had no effect on production of either inflorescences or laterals.

On 20 December bulbils that had formed no leaves were selected from plants grown under glass during winter. Six bulbils in each of two $3\frac{1}{2}$ in. pots were selected for each treatment and four treatments were given. Unfortunately the number of bulbils selected was insufficient because in each of three treatments only two germinated and from the other, three germinated, the rest remained dormant. The treatments were:

1. Short Day	(SD)	Cold Night	(CN)
2. Long Day	(LD)	,,	(CN)
3. Short Day	(SD)	Warm Night	(WN)
4. Long Day	(LD)	,,	(WN)

The S.D. was 8 hours while the L.D. was approximately 15 hours. S.D., C.N. plants had 16 hours of darkness at a temperature of 36°F. but the L.D., C.N. ones had only the first four night hours at this temperature, the rest of the time they were kept in darkness at normal night temperature.

Of the bulbs which germinated, those in treatments which included W.N. did so soonest and subsequent growth was more vigorous. All plants by late March had flowered and, as with the former experiment, no differences could be detected in the number of bulbils formed or their dormancy – most of these bulbils had, in fact, sent up leaves. Cold night temperatures alone as used in this experiment, are not sufficient therefore to induce dormancy in the bulbils.

GROWTH OF *O. latifolia* AS RELATED TO CONTROL MEASURES

Factors which are of significance in control are as follows: firstly factors liable to maintain the resistance of the plant. (A) A great proportion of the plant is below ground and the leaves and inflorescences which are accessible to the spray are present for only six months of the year. (B) Rate of growth of the bulb itself is slow and therefore it is not likely to be actively metabolising. Thus translocatable sprays are liable to be diverted to organs such as rapidly developing leaves, inflorescences and bulbils while the bulb may receive little. (C) Bulbils, unless they have produced leaves or are actively developing and receiving most of their nutrients from the mother bulb, are liable to be unaffected by translocatable sprays. (D) Bulbil production from a vigorous bulb is prolific. (E) Dormancy of bulbs and bulbils is a problem, especially when induced in the summer through drought, etc. The inaccessibility of these dormant bulbs and bulbils is a major problem in the control

of *Oxalis*. Secondly, relation of these aspects to control measures used are as follows. (A) Kill of all plant parts can theoretically be attained with soil sterilants. This has disadvantages such as high cost, the necessity of removing desirable plants in the vicinity and resultant soil toxicity. (B) Smothering, grassing down or periodic destruction of top growth by contact sprays has obvious disadvantages. Two seasons, maybe more, of such practices are needed to ensure all dormant bulbs and bulbils have been killed. (C) Translocatable sprays of the hormone type kill only a proportion of bulbs and bulbils. Other materials have been and will be tried but even if proved successful further growth from bulbs and bulbils, dormant at the time of spraying, would necessitate repeated sprays.

A problem still remains, perhaps the greatest hope is for the discovery of new chemical weedicides: sterilants which are cheap and quickly lost from the soil or translocatable sprays which are more effective on the bulb and bulbils.

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NOTE ON *STYLOCHUS ZANZIBARICUS* LAIDLAW, (TURBELLARIA, POLYCLADIDA), A SUSPECTED PREDATOR OF BARNACLES IN THE PORT OF AUCKLAND, NEW ZEALAND

By T. M. SKERMAN, New Zealand Oceanographic Institute, Department of Scientific and Industrial Research, Wellington.

(Received for publication, 14 July 1960)

Summary

Numerous superficially intact shells of the barnacles, *Balanus amphitrite cirratus* Darwin, *B. trigonis* Darwin, and *Elminius modestus* Darwin, attached to submerged test panels and ships' bottoms in Auckland Harbour have been found to be occupied solely by a polyclad, *Stylochus zanzibaricus* Laidlaw. Circumstantial evidence indicates that the stylochids are selectively preying upon living barnacles, but it has not been possible to confirm this suggestion because of difficulties in observing their behaviour in the field and in the laboratory.

During investigations of marine fouling organisms in the Port of Auckland (Skerman 1959) it was noticed that in summer months, many shells of the barnacles, *Balanus amphitrite cirratus, B. trigonis,* and *Elminius modestus,* attached to perspex test panels were inhabited solely by living polyclad turbellarians. In most cases these flatworms were coiled up and occupied the entire space within the barnacle shells of which the opercular plates were usually found intact (Fig. 1). Usually one, but sometimes two flatworms

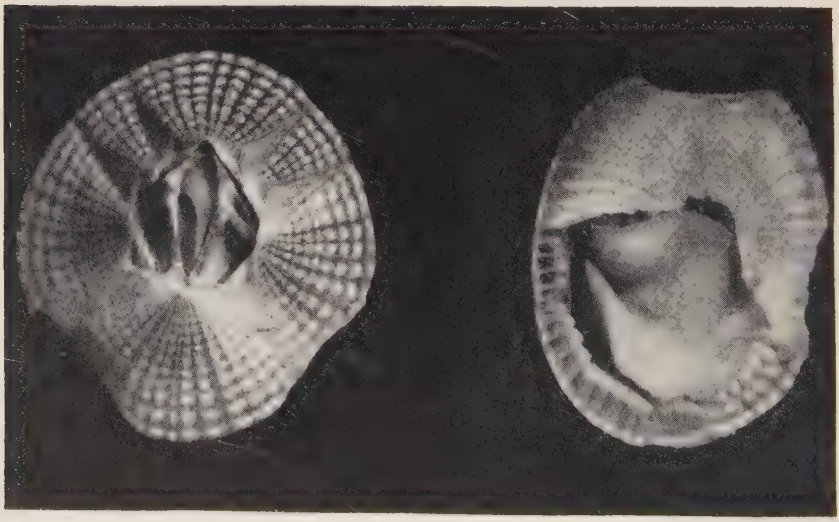

FIG. 1—*S. zanzibaricus* occupying a shell of *Balanus amphitrite cirratus.* Views showing *left* the body of the stylochid between the parted opercular valves, and *right,* its ventral surface after breaking open the basis of the barnacle. The protruded plicate pharynx of the flatworm can be seen on the mid-ventral surface. × 5.

could be found in each occupied shell. They were also commonly found roving freely over the crests of barnacles on panels exposed for periods up to 7 or 8 weeks, but were rare beyond this time when extensive epizoic growth of polyzoa and hydroids had enveloped and largely replaced the barnacle populations. The smallest barnacles found to be invaded were 5·0 mm in rostro-carinal diameter in *Balanus amphitrite,* the species most frequently affected, and 4·2 mm in *Elminius*: these were from a panel submerged for 3 weeks in December–January 1955. Panels immersed for longer periods, up to 2 months, bore correspondingly larger barnacles; greater proportions of these populations consisted of invaded shells. The flatworms were found associated with these barnacle-encrusted panels from late November to March – this is also the normal period of annual settlement in *Balanus* and the period of peak settlement in *Elminius* (Skerman 1959). They were more abundant on panels set at Kauri Point than at stations nearer the harbour entrance, corresponding with the conspicuously heavier barnacle settlement observed in the upper reaches of the harbour.

The polyclads, the largest mature specimen of which was taken live from a shell of *B. trigonis* and measured 22 mm in length and 8 mm in width when fully extended, have dark brown markings upon a greyish-white ground on the dorsal surface (Fig. 2); ventrally the colour is uniformly light grey. Through the kind cooperation of Dr Libbie Hyman of the American Museum of Natural History, they have been identified as *Stylochus zanzibaricus* Laidlaw. In his brief original description of this animal, Laidlaw (1903) noted that the specimens he examined from British East Africa were labelled "s.s. Juba". It is thus possible that the material was collected from or near a ship that may have been fouled by barnacles. Both Laidlaw and later, Meixner (1907) admit that the copulatory apparatus of *S. zanzibaricus* is identical to that of *S. neapolitanus,* described from the Mediterranean by Lang (1884), and that the two species are probably identical. Lang stated that *S. neapolitanus* were found abundantly inside the empty shells of barnacles ("Balanus – Colonien", p. 449).

Like the majority of turbellaria, stylochids are typically carnivorous. One form, *S. frontalis,* has been regarded as a serious pest in the oyster industry of the south east coast of the U.S.A. where it preys, apparently exclusively, on live oysters, eventually consuming the animals after gaining entry between the shell valves (Pearse and Wharton, 1938). In the present instance, however, unequivocal evidence of active predation of the barnacles has not yet been obtained. Careful microscopic examination of the contents of more than 50 invaded *Balanus* shells revealed no recognisable remains of barnacle tissue other than the opercular membrane. Not even chitinous remnants of barnacle exoskeleton were seen. In many instances, not only where pairs of worms occupied shells, sheets of encapsulated eggs lined the inner walls of the compartments, the basis, and the interior faces of the opercular valves (Fig. 3).

In field observations of stylochids clinging to barnacle-fouled panels placed in a dish of sea water, they showed little movement whatsoever, even in subdued light, and made no approach towards the barnacles which continued to display cirral activity. Captured stylochids could not be induced to

Photo: H. D. O'Kane

FIG. 2—A group of live *S. zanzibaricus* recently removed from barnacle shells. × 7.

feed in the laboratory either on minced fresh liver or on cut up portions of barnacle soft parts. The worms invariably died within 3 days of their removal from panels or *Balanus* shells and ensuing autolysis of the bodies left mere patches of grey slime at the bottom of culture dishes after 24 hours.

The fact that invaded barnacle shells were found among young *Balanus* populations in which mortality due to crowding, heavy epizoic growth or excessive silting of panel surfaces is likely to have been low, infers a probability that the barnacles were alive before the stylochids entered their

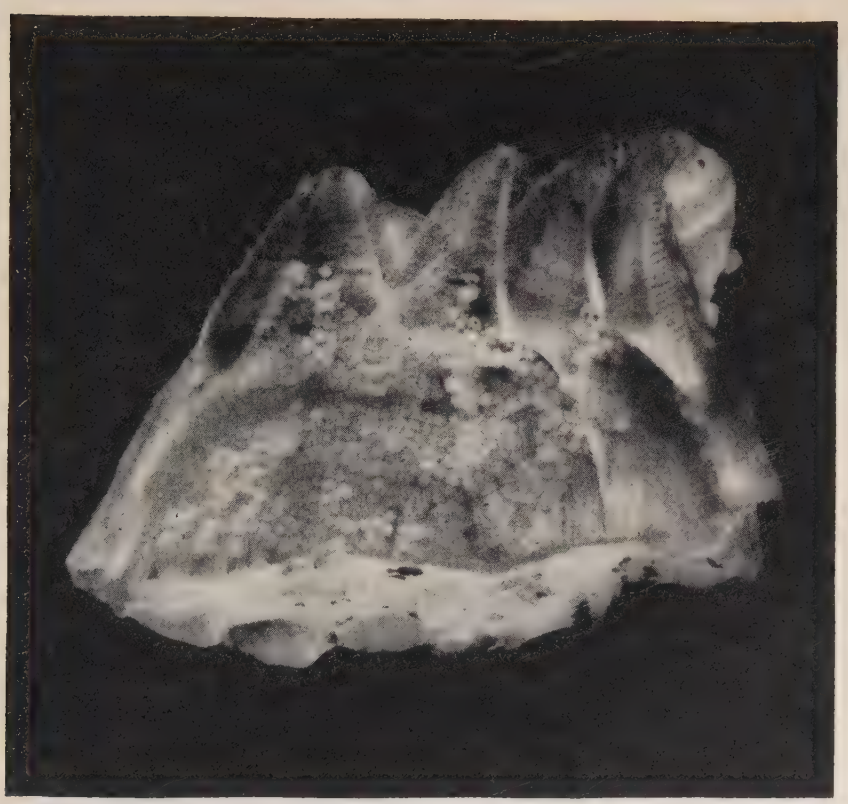

Photo: H. D. O'Kane

FIG. 3—Interior view of a *Balanus amphitrite* shell after removal of a pair of mature stylochids, showing egg capsules lining the inner walls of the compartments, sheath and basis. × 9.

shells — a view that is reinforced by the presence of intact opercular valves. Within this age group of barnacles there would have been few empty shells present had the stylochids been seeking solely a sheltered niche in which to lay eggs or complete some phase of the reproductive cycle.

It is not known whether the stylochids first became associated with test panels as free living larvae or as adults, derived perhaps from nearby wharf piles or other submerged structures. Their widespread incidence throughout the harbour in barnacle shells on test panels and also their appearance in samples of fouling barnacles from ships (Skerman 1960) would seem to support the former possibility. The smallest stylochid found free on a panel was 3·0 mm in extended length, that from within a barnacle shell 4·0 mm. Clearly the opercular opening provides the sole way of entry into the barnacle shell and possibly the stage when penetration is effected is influenced by the relative sizes of this opening and of the invading stylochid; this could explain why much higher proportions of *Balanus* populations were affected

than of the smaller, slower-growing *Elminius*. Selective predatory activity of the stylochids would require some preliminary searching behaviour and if, as a result, a suitable barnacle were encountered, an excretion of enzymes causing a relaxation of adductor and depressor muscles of the opercular apparatus might permit entry within the mantle cavity whereafter digestion of the contents could proceed. Cross fertilisation between polyclads may take place in cases of multiple entry of the same shell which would provide a secluded environment for the development of eggs subsequently laid.

Confirmatory evidence of selective predatory habits as suggested above is likely to prove difficult to secure in such an apparently capricious laboratory subject as *S. zanzibaricus*. Unfortunately the turbidity of Auckland harbour waters makes underwater observations of stylochids and barnacle populations almost impracticable. Nevertheless, the widespread occurrence on various submerged surfaces in the harbour of invaded shells of barnacles, few of which when alive would have attained maturity, indicates that *S. zanzibaricus* may be a factor having a substantial control on populations of fouling barnacles in this locality.

ACKNOWLEDGMENTS

For identifying the polyclad, and for providing helpful information the author's thanks are extended to Dr. Libbie Hyman of the American Museum of Natural History, New York. The help of members of the staff of the Drawing Office, H.M.N.Z. Dockyard, Devonport, Auckland, in securing additional material is greatly appreciated.

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THE RECENT ESTABLISHMENT OF THE POLYZOAN, *WATERSIPORA CUCULLATA* (BUSK), IN AUCKLAND HARBOUR, NEW ZEALAND

By T. M. SKERMAN, New Zealand Oceanographic Institute, Department of Scientific and Industrial Research, Wellington.

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Summary

Extensive establishment of encrusting colonies of the fouling polyzoan, *Watersipora cucullata* (Busk), on permanently submerged and intertidal surfaces within Auckland Harbour, New Zealand, is described with evidence suggesting this has taken place between the summers of 1955–56 and 1958–59. The origin of these populations of *Watersipora cucullata,* a species not previously recognised in New Zealand, is discussed. Overseas shipping is considered to have been the mode of introduction.

The cheilostomatous polyzoan, *Watersipora cucullata* (Busk), has been credited with a wide distribution in tropical and sub-tropical regions. Osburn, for instance, in his 1952 monograph, listed records of its occurrence in the Mediterranean, Red, Arabian, China, and Carribean Seas, the Indian Ocean, Gulf of Mexico, South Africa, Brazil, Colombia, and the Galapagos Is. *Watersipora* has also been long recognised as a ship-fouling organism (Hentschel 1923) in which capacity Weiss (1947) found it would readily settle and grow even on moderately toxic anti-fouling paints incorporating copper poisons.

Following World War II, investigations in Australian waters (Allen and Wood, 1950) have shown that *Watersipora cucullata* is prominent among ship-fouling organisms in Sydney Harbour. Because more general searches revealed it to be the most abundant shallow water polyzoan on the New South Wales coast – a fact not documented in any earlier faunal surveys – Allen (1953) considered that the species may have been recently introduced to the Australian region and suggested this may have been effected by ship transport. In support of this, Allen noted that, although known to the north and west of Australia, *Watersipora* had appeared only sporadically in intermediate localities.

No records exist of *Watersipora* being found on New Zealand coasts, nor did this species feature on test panels exposed in major ports (Skerman 1958b, 1959) or on shipping confined to the coastal trade (Skerman, 1960) in studies made between 1954 and 1956 on ship-fouling organisms of New Zealand waters. It was recognised, however, as a common fouling species on vessels trading between New Zealand and East Australian ports north of Port Kembla (Skerman 1960). Intact living colonies were taken from those ships docking in New Zealand after discharging their Australian cargo in summer, but only the skeletal parts remained of zoaria attached to ships dry-docking in winter in ports other than Auckland. From these observations, and from similarities in respect to broad ecological conditions and

species of fouling organisms in the sub-tropical ports of Auckland and Sydney, the spread of *Watersipora* to Auckland became a contingency that could not be disregarded (Skerman 1959).

On 15 March 1960, the author had the opportunity of examining raft installations set up in Auckland Harbour as part of an anti-fouling paint testing programme being carried out by the Royal New Zealand Navy on behalf of the Admiralty. From a "Bakelite" non-toxic control panel, a painted metal panel and a piece of wooden framing, all submerged at depths less than 4 ft, were collected three encrusting polyzoan colonies averaging 2 in. in diameter. These proved to be *Watersipora cucullata.* Later, an examination at low tide of nearby wharf piles in the vicinity of the Devonport Naval Base revealed much larger colonies, 5 to 8 in. in diameter, growing upon horizontal concrete beams which, at the time, were exposed some 18 in. above the water. Eighteen such colonies were counted on one side of a cross beam, some 14 ft in length and 12 in. by 16 in. in section. The following day, a naval vessel that had been laid up in Auckland Harbour for the past 20 months entered dry dock, and was found to bear numerous *Watersipora* colonies on underwater plates to a least depth of 3 ft below the waterline.

Inspection of the illustrated records of the current Navy paint-testing programme which was commenced in December 1958, shows that *Watersipora* colonies were present on a panel examined on 23 April 1959 after 4 months' immersion. Other panels, examined at later dates, corroborate this evidence of settlement during the 1958–59 summer, and as late as May, but it is not clear whether settlement continued during the winter months of 1959. The author's series of test panel exposures at the Devonport Naval Base (Station A4, Skerman 1959) which failed to demonstrate *Watersipora* as a fouling growth inhabiting Auckland waters, concluded in the summer of 1955–56. Several large colonies are now growing on wharf piles within 2 ft of the original test site. It appears, then, that the species became extensively established on permanently submerged surfaces and on surfaces at lower levels of the inter-tidal zone, at least in this area of Auckland Harbour, between the summers of 1955–56 and 1958–59. Undetected pioneer colonies, precursors of present populations and perhaps only few in number, may have been present prior to this.

The evidence of fouling samples from Trans-Tasman vessels strongly implicates shipping as the mode of introduction of *Watersipora* to New Zealand waters, since larval dispersal by ocean currents to such an isolated land mass would seem inconsistent with Wisely's (1958) experimental observations of larvae settling within 24 hours of liberation from parent colonies. It remains to be established whether the invasion by *Watersipora* of Auckland Harbour, which appears on the foregoing evidence to have been a recent event, has followed from an initial introduction to the port by shipping or from its spread from adjacent coasts already colonised, possibly north or east of Auckland, where it may have been introduced some time earlier. Yet, of a number of reports on intertidal communities on the east coast and off-lying islands of the North Auckland Peninsula (Cranwell and Moore, 1938, Carnahan 1952, Dellow 1955) none record observations of the conspicuous colonies of *Watersipora* with their typical dark purple to black centres and bright flame coloured frilled edges. However, the author

has been informed by Mrs P. Bergquist, University of Auckland, (pers. comm.) that encrusting colonies answering this description were observed by her on Kawau Is. (Hauraki Gulf) in late 1953 and on Rangitoto Is. in 1956. Although unconfirmed by an examination of material, the former record would pre-date the widespread appearance of *Watersipora* in Auckland Harbour and it raises the possibility of some other port being the original site of its introduction to the country. Whangarei, lying some 60 miles farther north, is the only port north of Auckland open to overseas shipping, but the volume of such traffic is relatively low (24 entries in 1956 compared with 627 at Auckland*). Until the occurrence of *Watersipora* on coasts north of Auckland is investigated more fully this question cannot be resolved further, but the possibility cannot be dismissed that coasts beyond Auckland Harbour, perhaps in providing a more appropriate environment, might have been colonised more rapidly by current-borne larvae released from a small number of pioneer colonies already established within the harbour itself.

It would be hazardous to suggest the probable country of origin of the introduced *Watersipora* populations. The author (1960) examined fouling samples from only few overseas ships other than those from Australia, and it must be conceded that a wide range of overseas shipping traffic may carry colonies capable of liberating larvae in Auckland waters. Further, ultimate colonisation may not necessarily be traceable to a single event; immigrant colonies on ships of diverse ports of origin on many occasions are more likely to have been, and undoubtedly still are, involved. Nevertheless overseas shipping entering New Zealand ports from Australia exceeds that from any other country (in 1956, from Australia 38% of the total, United Kingdom and Europe 22%, Pacific Is. 18%, North America 8%, Japan and Indonesia 4%, others 6%*). A further point favouring an Australian origin is the similarity between the operculum of New Zealand specimens and that figured by Wisely (*op. cit.*) for material from Sydney Harbour. Figure 1 illustrates the shape of the aperture, prominent condyles, and dark sclerites of the operculum, in which characteristics there is correspondence with the typical form, rather than with its described varieties, as discussed by Hastings (1929) and Mawatari (1952).

The period of peak settlement by *Watersipora* in Sydney Harbour is described by Wisely (*op, cit.*) as from February to May, but he found settling at reduced intensity continued throughout the winter. In the Port of Auckland, possibly the most substantial barrier to establishment of the species would be the winter minimum sea temperatures. In 1954 and 1955, the lowest monthly average temperatures occurred in August (11·4°C) and July (11·3°C) respectively, the extreme lowest recordings for these months being 8·7°C and 10·6°C (Skerman 1958a). For Sydney Harbour, Wisely (1959) gives lowest monthly averages of 14·8°C (August 1955) and 14·7°C (July 1956) with minima of 14·0°C and 14·1°C for recording taken between August 1955 and December 1956. This raises the question of whether, in the event of local *Watersipora* populations failing to survive the more severe Auckland winter temperatures, the harbour might not be stocked each sum-

*New Zealand Official Year-Book, 1958, Govt. Printer, Wellington.

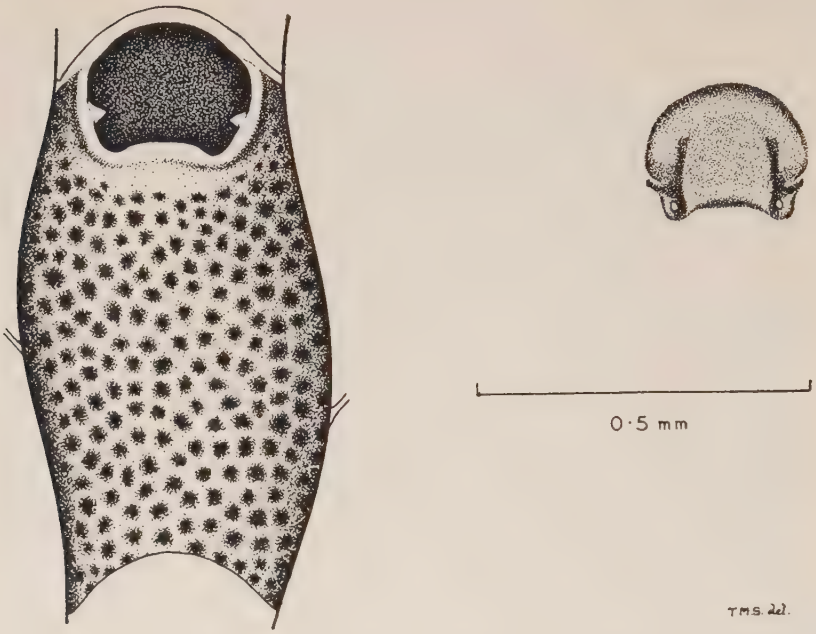

FIG. 1—Zooecium and form of aperture (left), and operculum (right) from a colony of *Watersipora cucullata* growing upon a test panel in Auckland Harbour.

mer from immigrant colonies on visiting ships, but from the size and numbers of existing encrusting zoaria on intertidal surfaces this possibility seems unlikely.

The impact of *Watersipora* as a ship-fouling organism in New Zealand waters may be considerable by virtue of its potential for growth on copper-containing anti-fouling paints. Attached colonies are reputed to grow rapidly, so providing a non-toxic substratum favourable for the settlement of more susceptible fouling species (Wood and Allen 1958). The experimental findings of Weiss (1947), recently confirmed by Wisely (1958), that the larvae are particularly sensitive to mercury suggest that traditional protective compositions in local use might require modification in the future.

ACKNOWLEDGMENTS

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SHIP-FOULING IN NEW ZEALAND WATERS: A SURVEY OF MARINE FOULING ORGANISMS FROM VESSELS OF THE COASTAL AND OVERSEAS TRADES

By T. M. SKERMAN, New Zealand Oceanographic Institute, Department of Scientific and Industrial Research, Wellington.

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Summary

The incidence and frequency of marine fouling organisms associated with ships trading to and within the New Zealand region are reported from the examination in dry dock of 89 vessels operating on a variety of routes.

On ships comprising the general coastal traffic the following species were prominent: Algae, *Enteromorpha* sp., *Ulva lactuca*; Hydroids, *Tubularia larynx, Phialella quadrata*; Serpulids, *Hydroides norvegica, Galeolaria hystrix*; Polyzoa, *Bugula neritina, B. flabellata, Cryptosula pallasiana*; Molluscs, *Mytilus edulis planulatus, Ostrea* sp. cf. *O. sinuata*; Barnacles, *Elminius modestus, Balanus amphitrite cirratus.* Fouling on vessels including in their itinerary ports of the Auckland region was distinguished by the presence of *Balanus amphitrite, B trigonis* and a relatively high incidence of *Bugula neritina, Hydroides, Tubularia* and oysters; ships confined to more southern waters more frequently bore algae, *Bugula flabellata, Cryptosula, Galeolaria* and mussels. *Elminius modestus* was the commonest single species encountered in both of these groups.

Species conspicuous on vessels engaged between New Zealand and East and South Australian ports included *Balanus amphitrite cirratus, Hydroides norvegica, Bugula neritina, Tubularia larynx, Elminius modestus,* and the encrusting polyzoan *Watersipora cucullata.* Heavy settlement by *Hydroides norvegica* was acquired by two vessels operating between New Zealand and Indian Ocean ports. Ships from tropical Pacific waters were marked by the presence of a hydroid *Pennaria* (?) sp., a polyzoan, *Schizoporella* sp., and the following barnacles, *Lepas anatifera, L. anserifera, Balanus amphitrite denticulata, B. auricoma* and *Chthamalus hembeli. Conchoderma auritum* and *C. virgatum* were the only organisms present on vessels that had been operating in Antarctic waters.

Fouling characteristics of the major groups of fouling organisms encountered are discussed together with features of sequences in the settlement of species, shell deformation in barnacles growing on toxic surfaces, and the anaerobic corrosion of steel plates.

INTRODUCTION

In years following the Second World War, certain shipowners operating in the Australasian trade became concerned over losses in speed and increases in fuel consumption that were attributed to the unusually fouled condition of the underwater plating of their vessels. Visits by ships to ports in this region, it was claimed, were accompanied by fouling of a severity that customary brands of anti-fouling paints seemed unable to prevent, and although extended delays within these ports were common at the time, it was speculated that foreign marine fouling organisms of high resistance to paints of traditional toxicity might have become recently established in the region.

An inadequate background knowledge of the nature of marine biofouling in Australasia, or indeed, the Southern Hemisphere as a whole, precluded any immediate appraisal of these problems confronting the shipping trade, but soon prompted studies in various ports of Australia (Wood, 1950, 1955; Allen and Wood 1950, Wood and Allen 1958; Allen 1950, 1953; Wisely 1958a, 1958b, 1960a, 1960b), New Zealand (Skerman 1956, 1958a, 1958b, 1959), and South Africa (Millard 1952, 1959). From these observations, information has accrued concerning the identity, geographic distribution, seasonal abundance and settlement characteristics of many marine fouling organisms to which ships in these ports would be exposed.

With a view to an elucidation of the ship-fouling problem in New Zealand waters, and to corroborate experimental observations in ports (Skerman, *op. cit.*) a survey was planned of marine organisms fouling ships that had been operating in this region prior to dry-docking. Primary objectives were to establish the identity, frequency of occurrence and distribution of the predominant species associated with the various shipping trade routes.

Initially it was hoped that examination of a small number of selected vessels in dry dock would have yielded sufficient evidence of which fouling species were of primary local importance. This information was, however, so variable, according to the class and duties of the ships, their ports of call, and seasonal factors, that a considerable extension of the programme was demanded before any consistent picture of fouling trends became evident. This report summarises results from the examination of 89 ships from a variety of classes and duties that docked in New Zealand between 1954 and 1956.

METHODS

Graving docks located at Auckland, Lyttelton, and Port Chalmers, and a floating dock at Wellington are owned and operated by the local harbour authorities. Slip-way facilities of capacities varying from 50 to 500 tons deadweight are available at these and at a number of the smaller New Zealand ports. Compliance with survey regulations necessitates vessels in service to dock once every twelve months, but depending on the type of service they are engaged in, some vessels are docked for cleaning and repainting at shorter intervals. As a result of a heavy local demand upon existing docking facilities in New Zealand, few overseas-registered ships make use of them except in cases of emergency. Consequently, few vessels operating beyond the Australasian region became available for inspection.

Samples of fouling growths on each ship were collected from as many different areas of the hull as required for a representative sample. Usually a 2 lb jar was filled. By use of a questionnaire, information was recorded of the distribution of the predominant organisms on wind-and-water plates, side plates, bottom plates, and keel, condenser and fire-main intakes, rudder, propeller blades and boss, with a rough quantitative fouling rating of each. Previous painting history and general information on the state of steel and paint surfaces were also sought while details of the ship's movements during the recent out-of-dock period were obtained from company records.

Although any assessment of the fouling growth borne by a ship implies some evaluation of the anti-fouling performance of the paint used under the conditions to which it has been subjected, no systematic appraisal of painting compositions has been attempted.

DATA

As has been shown by previous workers (Bishop *et al.* 1949) each vessel entering dry dock presents a complex picture with regard to the fouling it bears. In some cases, detailed analysis may reveal the ports of origin of the respective fouling growths, their capacities for survival over different trade routes and information concerning the probable length of life of the anti-fouling composition used. In general practice, however, such an analysis is often hampered by the fact that untreated areas of a vessel's hull that may serve as non-toxic control surfaces are seldom available for comparative observations.

In addition to many hundreds of samples of fouling growths from ships, a substantial amount of supplementary data such as the identity, ownership, tonnage, and trade movements of vessels accumulated from this investigation. Because the aims were confined to a biological assessment of the problem, many of these details, pertinent only to the assembly of the data, have been omitted here.

One difficulty arising in a survey of this nature is the quantitative assessment of the fouling growth on a ship's hull. Various rating systems have been proposed (Pyefinch 1945, Bureau of Ships, 1944) mainly in assessment of the performance of the anti-fouling properties of different paints. Such procedures were considered unnecessary for the present purposes and a more subjective appraisal of fouling intensity was adopted. Thus, records show firstly the different species found on ships of various types and secondly, the number of these ships on which each species was regarded as an important component of the various fouling assemblages. Fouling is essentially a port phenomenon, and as a regional interpretation of ship-fouling according to the major trade routes followed has the most practical application, the above analysis has been assembled on these lines.

INCIDENCE OF FOULING SPECIES ON SHIPPING

Class 1. Ships Confined to Single Ports of New Zealand

In addition to vessels out of commission, tenders, dredges, floating cranes, and tugs spend a large proportion of their service period at rest and are therefore susceptible to the settlement and growth of many species. If intervals between docking and repainting are lengthy, fouling tends to be severe.

Ships from the following ports were examined.

AUCKLAND

All Royal New Zealand Navy vessels are based at this port. A number of frigates and minesweepers in permanent reserve provided an opportunity

of comparing fouling developing on ships with that arising on nearby test panels (Skerman 1959). These ships, of which 24 were examined, normally docked every 12 months.

WELLINGTON

Seven vessels, either in permanent service or laid up in Wellington Harbour, were examined.

OTHER PORTS

Three dredges, based at Napier, Dunedin, and Bluff were inspected in dock. Apart from the small number of cases involved, fouling from dredges is not considered to be truly representative of the home port, for the silt-laden waters about a dredge during operations probably impose some degree of selection on the survival of attached fouling organisms.

The incidence and frequency of species recorded from ships at these ports are summarised in Table 1.

It is a general experience that the prolonged immersion in the sea of a suitable substratum ultimately results in the development of a fouling assemblage, the structure of which is to some extent reproducible, stable, and characteristic of the locality observed. This has led some authors (e.g. Woods Hole Oceanographic Institution 1952, Millard 1952) to identify such assemblages as fouling communities (*sens. strict.*) taking their name from one or more of the predominant species. Typical groups of organisms, with many of the above mentioned properties, did tend to develop upon vessels in the category under consideration as a result of complete or partial immobility or extended out-of-dock periods. Thus molluscs and ascidians, both of which are slow to colonise newly-immersed surfaces, but which may become dominant on prolonged immersion, were conspicuous on ships in both Auckland and Wellington groups. Whereas oysters (*Ostrea*) and the ascidians *Microcosmos, Styela* and *Botrylloides* were major representatives of the Auckland group, mussels (*Perna* and *Mytilus*) and the ascidians *Diplosoma* and *Asterocarpa* were prominent in long-term Wellington fouling, showing good agreement with experimental records by Skerman (1959) and Ralph and Hurley (1952) respectively. Other distinguishing species were the hydroid *Tubularia,* the polyzoans *Bugula neritina* and *Caberea zelandica,* and the serpulid *Hydroides* that featured in Auckland fouling, whereas *Bugula flabellata* and *Cryptosula* were the most frequently recorded polyzoans at Wellington where the dominant serpulid was *Galeolaria hystrix.* Presumably, because of the generally advanced stage reached in the development of the respective fouling assemblages, barnacles, which usually number among the primary colonisers of surfaces, were not abundantly represented on vessels in this group. *Balanus amphitrite* and *B. trigonis* were exclusively restricted to the Auckland ships and the higher incidence of the common New Zealand shore species *Elminius modestus* at Wellington may have resulted from a lack of substantial competition from other barnacles at this port. *Balanus decorus* was recorded sporadically only from Wellington vessels although this barnacle is known to inhabit waters as far north as the Kermadec Is. (Linzey 1942).

TABLE 1—Incidence of Fouling Species on Ships Confined to New Zealand Ports (Class 1)

Fouling Species	AUCKLAND Total No. Ships = 23		WELLINGTON Total No. Ships = 7		NAPIER Total No. Ships = 1	DUNEDIN Total No. Ships = 1	BLUFF Total No. Ships = 1
	No. Ships Recorded From	No. Ships Recorded as Dominant	No. Ships Recorded From	No. Ships Recorded as Dominant			
ALGAE							
Enteromorpha intestinalis			2	0			
Ulva lactuca			2	0			1
PORIFERA							
Unident sp.	5	0	4	0			1
ACTINIARIA							
Unident sp.	7	0	1	0			
HYDROIDS							
Tubularia larynx	15	5	1	0			1
Phialella quadrata	3	0	1	0			
Halecium delicatulum	1	0					
Clytia (?) elongata	2	0					
Syncoryne tenella	1	0	1	0			
Turritopsis nutricola	2	0					
Obelia dichotoma			2				
Plumularia pulchella							1
POLYZOA							
Bugula neritina	23	15	5	2			
Bugula flabellata	3	2	6	1		1	1
Bugula avicularia	6	0					
Caberea zelandica	5	4					
Cryptosula pallasiana			4	1		1	1

SERPULIDS							
Hydroides norvegica	20	9	1	0		1	
Galeolaria hystrix			6	4		1	
Pomatoceros carciniferus			3				
MOLLUSCS							
Ostrea cf. *O. sinuata*	9	0	1	0	1		
Mytilus edulis aoteanus			2	2	1		
Perna canaliculus ..	9	0	7	2			
BARNACLES							
Balanus amphitrite cirratus	4	2					
Balanus amphitrite communis	5	1					
Balanus trigonis	7	1					
Balanus decorus			2	0	1		1
Elminius modestus .	12	6	5	3	1		
ASCIDIANS							
Microcosmos kura	7	1					
Asterocarpa cerea	10	3	1	0			1
Styela plicata	7	2					
Corella eumyota ...	8	2	2	0		1	
Botryllus schlosseri ...	3	0					1
Diplosoma macdonaldi	8	3	4	0		1	1
Botrylloides leachi	8	8					
Ciona intestinalis	1	0					
Pyura pachydermatina			2	0	1	1	1
Aplidium (Amaroucium) phortax	1	0					

As settlement of the majority of fouling organisms recorded at both Auckland and Wellington follows seasonal patterns, differences in the composition of fouling would be expected between ships according to the particular seasons spent out of dock. Any such effect, however, would be masked when the service period extends over one or more seasonal cycles. Too few ships at Wellington were examined to reveal any outstanding seasonal differences, and at Auckland where most of the predominant fouling species are capable of attaching from September nearly to May (Skerman 1959), the average out-of-dock period of 12 months provided the opportunity for a vessel to acquire all representative species. However, those of the Auckland ships that docked for repainting during the first half of the year showed much heavier, recent, *Balanus* settlements (*B. amphitrite* and *B. trigonis*) than did those docking in later months. A similar trend was noted in regard to oysters. Thus, anti-fouling compositions applied in the spring appeared to withhold barnacle colonisation during the on-coming summer, but when applied in the autumn, could not afford similar protection in the following summer at the end of the out-of-dock period. No seasonal differences arose in the occurrence of the forms *Tubularia, Hydroides,* and *Bugula neritina,* all of which settle over a longer period of the summer than do the above mentioned barnacles.

Class 2. New Zealand Coastal Shipping

This category includes 31 examined vessels that had been operating exclusively on the coastal trade. Of these, some were engaged on round voyages which included calls at all ports from Auckland to Bluff. Other traffic, such as passenger express vessels, had restricted movements between two ports only, and further distinction could be made between vessels trading solely in North Island and South Island waters respectively. For the majority of vessels considered in this class, docking took place at approximately 12 month intervals.

Figures giving the incidence of different fouling species on all coastal vessels are recorded in Table 2. The first column lists the number of ships on which each species was observed while the number of separate occasions on which each species was a dominant fouling form appears in the second column. As it became clear that the fouling characteristics of ships that had included Auckland as a port of call differed qualitatively from those occupied on more southern routes, the incidence of species on ships divided between these categories has been also tabled in the 3rd and 4th columns respectively, figures in bold type appearing under the route in which each species was recorded most frequently. Other subdivisions can be made according to specified internal trade routes, but as relatively few ships were involved they are discussed in the text.

Qualitative comparison of these records with those from port shipping (Table 1) shows the lower incidence of the slow-establishing ascidian component of fouling on vessels in regular service. The effects of including Auckland as a port of call are also marked, for whereas algae, mussels, polyzoa as represented by *Bugula flabellata* and *Cryptosula, Galeolaria, Elminius* and the hydroid *Phialella quadrata* typified fouling on ships confined to southern routes, oysters, *Bugula neritina, Hyroides, Tubularia,* and

barnacles represented by *Elminius* and exclusively by *Balanus amphitrite* and *B. trigonis,* characterised fouling on round voyages that included Auckland. On this basis, with an out-of-dock period of about 12 months (which therefore covered at least one seasonal cycle of all settling organisms) it became possible to certify with some precision from inspection of the fouling alone which of these two overlapping zones a vessel had been operating in, not from the evidence afforded by a single species alone (*Balanus* excepted) but from the general structure of the fouling assemblage.

TABLE 2—Incidence and General Distribution of Fouling Species from Coastal New Zealand Shipping (Class 2)

(Total Number of Ships Examined, 31)

Species	No. Ships Recorded	No. Ships Recorded as Dominant	Frequency on Major Trade Routes	
			All Ports, Including Auckland (17)	Ports South of Auckland (14)
ALGAE				
Enteromorpha sp.	8	5	2	**6**
Ulva lactuca	7	1	2	**5**
ACTINIARIA				
Unident sp.	3	0	**2**	1
HYDROIDS				
Tubularia larynx	12	8	**8**	4
Syncoryne tenella	2	0	1	1
POLYZOA				
Bugula neritina	10	6	**8**	2
Bugula flabellata	8	2	4	**4**
Cryptosula pallasiana	11	6	5	**6**
Caberea zelandica	1	0	**1**	0
SERPULIDS				
Hydroides norvegica	10	7	**6**	4
Galeolaria hystrix	5	2	1	**4**
Pomatoceros carciniferus	3	1	1	**2**
MOLLUSCS				
Ostrea cf. *O. sinuata*	8	4	**6**	2
Mytilus edulis aoteanus	15	5	6	**9**
Perna canaliculus	5	1	1	**4**
Anomia walteri	1	0	**1**	0
BARNACLES				
Balanus amphitrite cirratus	8	4	**8**	0
Balanus amphitrite communis	5	4	**5**	0
Balanus trigonis	3	2	**2**	0
Balanus decorus	3	0	0	**3**
Lepas australis	1	0	0	**1**
Elminius modestus	29	23	16	13
ASCIDIANS				
Microcosmos kura	2	0	**2**	0
Styela plicata	1	0	**1**	0
Botryllus schlosseri	3	0	2	1
Asterocarpa cerea	2	0	0	**2**
Pyura pachydermatina	2	0	0	2

Regular trade routes within this general category will be discussed separately.

2a. Steamer Express Service, Wellington–Lyttelton

Two vessels sail on this route on six nights of the week and are berthed at the terminal ports during alternate days. They were examined in dock on six occasions, one ship four times and the second, twice. According to the exigencies of the service, vessels were docked at either 6 or 12 monthly intervals, in the latter cases anti-fouling paints of higher toxic content being applied. Fouling species recorded on these vessels are listed in Table 3.

TABLE 3—Incidence of Fouling Species from Wellington-Lyttelton Passenger Steamers (Class 2a)

(Total number examined, 6)

Species	No. Ships Recorded	Species	No. Ships Recorded
ALGAE		SERPULIDS	
Enteromorpha intestinalis	4	*Hydroides norvegica*	2
Ulva lactuca	2	*Galeolaria hystrix*	3
		Pomatoceros carciniferus	1
ACTINIARIA			
Unident sp.	1	MOLLUSCS	
		Ostrea sp. of *O. sinuata*	2
HYDROIDS		*Mytilus edulis aoteanus*	5
Tubularia larynx	3	*Perna canaliculus*	2
Obelia australis	1		
Phialella quadrata	1	BARNACLES	
		Balanus decorus	2
POLYZOA		*Elminius modestus*	5
Bugula neritina	2		
Bugula flabellata	3	ASCIDIANS	
Cryptosula pallasiana	3	*Botryllus schlosseri*	1
		Asterocarpa cerea	1
		Pyura pachydermatina	1

Presumably as a result of the relatively large proportion of the service period spent in port, fouling on these vessels tended to be heavier than on ships spending longer periods under way. The composition of the fouling developed was typical of the "southern complex" outlined above. Some elements, however, were peculiar to vessels in this service. Due to the small variation between heavy and light load lines, green seaweed fouling, which arose particularly during the spring, tended to be retained and because these ships regularly berth port side to at both termini the alga *Enteromorpha* became concentrated by the waterline on starboard plates exposed to the light. This was reported to have an appreciable effect upon the manoeuverability of the ships while berthing. Mussel fouling (*Mytilus*) was also particularly severe within and about the openings of ducts leading to domestic and condenser water systems. Other species notably in abundance were the encrusting polyzoan *Cryptosula* and the tubeworm *Galeolaria hystrix*.

2b. Wellington –Westport/Greymouth

The one vessel permanently operating on this route has been singled out because the latter two ports are situated within the mouths of rivers. Only four species were present on the ship after an out-of-dock period of 13 months. Waterline fouling was heavy and was composed of *Enteromorpha. Elminius* barnacles were widely distributed on most underwater plates while mussels (*Mytilus*) were concentrated on bottom areas. It is likely that the paucity of species on this vessel after more than a year in service resulted from the time spent in estuarine ports. *Elminius modestus* is known to thrive over a wide range of salinities; mussels, in being confined to the lower regions of the hull, may have found more suitable saline conditions at this depth.

2c. Ports including Wanganui

A vessel more than a year out of dock having made regular calls at Wanganui, a river port, bore heavy settlements of *Elminius modestus* as the sole fouling organism. This instance further illustrates the low salinity tolerance of this form and the selective effect of such a port on the survival of fouling growths.

2d. Onehunga – Tasman Bay Ports

No information is available on fouling organisms present in the port of Onehunga within the Manakau Harbour. In summer months, however, it appears that settlement by *Balanus amphitrite* is heavy. One vessel on the above route was examined on four successive dockings which are considered here on account of the seasonal variation in fouling that was recorded. Docking was approximately 6-monthly, late spring and late autumn.

At each docking of this ship, fouling by barnacles was found to be conspicuously heavy. (Fig. 1.) Both *Elminius modestus* and *Balanus amphitrite cirratus* appeared after service during the summer period, but only the former was present at the time of the spring dockings. The sparse representation of other growths which included mussels and *Tubularia,* may have been an effect of low salinities encountered at the ports of Onehunga and Motueka.

Class 3. Trans-Tasman Shipping

Thirteen separate inspections were made of 11 cargo ships operating between New Zealand ports and those on the south and east coasts of Australia. In most cases, out-of-dock periods were about 6 months, but three vessels were examined after nearly 12 months' service. The number of round voyages completed (varying from 3 to 8) depended upon the number of ports of call involved in each.

Records of the fouling species associated with these vessels are summarised in Table 4.

FIG 1—Heavy fouling exclusively by the barnacles *Balanus amphitrite cirratus* and *Elminius modestus* on a ship trading between the ports of Onehunga and Motueka.

Experimental records in ports of both Australia and New Zealand have revealed many species of fouling organisms to be common to both regions. Similarities both in regard to species observed and their seasons of settlements are particularly marked between Sydney and Auckland (cf. Allen and Wood 1950, and Skerman 1959). Sea temperatures in Sydney Harbour

appear to be some 3° to 4°C higher on the monthly average than in Auckland Harbour (Wisely 1959, Skerman 1958a). Thus the high incidence of *Balanus amphitrite cirratus, Hydroides norvegica, Bugula neritina,* and to a rather less extent, *Tubularia,* as fouling organisms on trans-Tasman ships could be attributed to the fact that settlement in all four species may take place in both New Zealand (Auckland) and East Australian ports of call.

TABLE 4—Incidence and Abundance of Fouling Species from Cargo Vessels Operating Between New Zealand and Australian Ports (Class 3)

(Total number of ships examined, 13)

Species	No. Ships Recorded	No. Ships Recorded as Dominant	Species	No. Ships Recorded	No. Ships Recorded as Dominant
ALGAE			MOLLUSCS		
Enteromorpha sp.	2	0	*Ostrea* sp.	6	0
Ulva lactuca	1	0	*Mytilus edulis*	6	0
			Perna canaliculis	2	0
HYDROIDS			*Anomia walteri*	1	0
Tubularia larynx	7	3			
Syncoryne tenella	2	0	BARNACLES		
			Balanus amphitrite cirratus	11	10
POLYZOA			*Balanus amphitrite communis*	2	0
Bugula neritina	8	3	*Balanus trigonis*	1	1
Bugula flabellata	3	1	*Elminius modestus*	8	6
Bugula avicularia	3	1			
Cryptosula pallasiana	3	1	ASCIDIANS		
Watersipora cucullata	5	4	*Microcosmos kura*	1	0
			Styela plicata	2	0
SERPULIDS			*Ciona intestinalis*	1	0
Hydroides norvegica	11	7	*Asterocarpa cerea*	1	0
			Botryllus schlosseri	2	0

Some differences in the species composition of fouling were noted between vessels trading between New Zealand and South Australian ports and those trading between New Zealand and East Australian ports north of Port Kembla. The former group bore appreciably denser populations of *Elminius* barnacles than of *Balanus* and the encrusting polyzoan, *Cryptosula* was also common. In the latter group of vessels, 5 of a total of 9 bore the encrusting polyzoan *Watersipora cucullata* and all carried fairly heavy settlements of *Balanus amphitrite cirratus.* Wood and Allen (1958) record *Watersipora* as occurring on all Australian coasts except those of Victoria and Tasmania. In view of this, it was of interest to observe that only the skeletal parts of encrusting colonies were found on vessels after completing the discharge of Australian cargo in New Zealand ports south of Wellington in the winter. Vessels docking at these ports in summer, and one ship docking in Auckland in winter, were found to carry *Watersipora* colonies that were intact and apparently alive at the time of collection. It is possible that

Watersipora fails to survive journeys into waters dominated by subantarctic influences, the same barrier withholding its spread on the Australian coast further south than New South Wales.

In recent revisions, Powell (1958) and Fleming (1959) have distinguished the common mussels of southern New Zealand and of South Australian coasts as subspecies (*aoteanus* Powell and *planulatus* Lamarck respectively) of *Mytilus edulis* L. Few mussels were taken from ships in the New Zealand – Australian service and unfortunately their small size precludes their assignment to one or the other subspecies as characterised by the above authors. Wood and Allen (1958) record *Mytilus planulatus* (cf. *Mytilus edulis planulatus*) as a common fouling mussel in southern Australian waters, and the possibility that trans-Tasman shipping may offer an interchange between *Mytilus* populations of these and New Zealand coasts must be borne in mind.

Class 4. New Zealand – Indonesia – Ceylon

Two vessels regularly operating on this route were inspected. Following loading or discharging cargo in ports of both islands of New Zealand, calls were made at Singapore and Port Swettenham in Malaya, Calcutta, and on the return voyage from Colombo at Semarang and Sourabaya in Indonesia. One ship had completed 3 round voyages before docking (10 months out of dock), the other only one (8 months out of dock).

The first ship was heavily fouled primarily by serpulids (*Hydroides norvegica*) and barnacles which included *Elminius modestus, Balanus trigonis, B. amphitrite communis* and a second unidentified subspecies of *B. amphitrite.* All barnacles except *Elminius* were dead when collected in Wellington dock. Figure 2 illustrates the intensity of *Hydroides* settlement, to which the serious losses in propulsive efficiency reported from this vessel can no doubt be ascribed.

Calcareous tubes of *Hydroides* were also a conspicuous component of fouling on the second vessel which docked at Pt. Chalmers. Water-line plates carried in addition a small amount of weed (*Enteromorpha* and *Ulva*) while broken stems of the hydroid *Tubularia* were common on lower plates. Barnacles, less abundant than on the first ship, included *Lepas anserifera* L., generally recognised as an inhabitant of tropical waters, *Balanus amphitrite communis* and *B. a. amphitrite.*

Ships on this route appear to be particularly susceptible to *Hydroides* fouling. This tubeworm has been reported from Madras Harbour (Paul 1942) and Visakhapatnam Harbour (Ganapati *et al.* 1958) in India and it seems likely that it may settle in most of the ports of call as well as in New Zealand. Thus it is unlikely that growth is interrupted by unfavourable conditions at any of the ports of call once settlement has taken place. *B. amphitrite communis* is generally well known in tropical and sub-tropical regions and has been reported from Indian waters by the latter authors. *B. trigonis* records of Darwin (1854) included Java and the East Indian Archipelago.

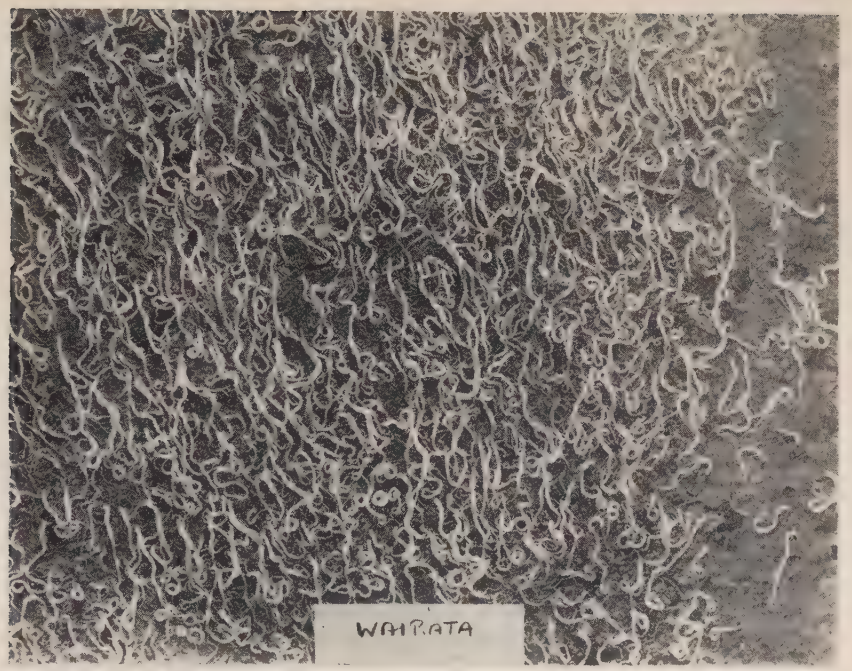

FIG. 2—Intensive settlement by the tubeworm *Hydroides norvegica* acquired by a ship trading between New Zealand and Ceylon via Indonesia.

It could not be concluded whether the *Elminius* barnacles on the first ship had survived a round voyage to and from Indian waters or had settled in home waters prior to docking. Specimens were of a size (5–6 mm basal diameter) that experimental observations indicate could have been attained at the end of a month's growth after settlement in summer at Auckland (Skerman 1959), and 36 days had been spent in this port discharging cargo before proceeding to dock at Wellington. On the other hand, it is known that growth is slow in barnacles when ships carrying them are under way, due to the restrictions imposed on feeding processes, and these adult specimens could equally well have been much older than one month.

Class 5. Shipping from the Tropical Pacific

Three vessels operating in tropical waters of the Central and South West Pacific Ocean, or between here and New Zealand were available for examination. None bore a great deal of growth, but the species recorded from them are listed below in Table 5.

TABLE 5—List of Species Recorded from Three Vessels Operating in Tropical Waters of the Central and South-west Pacific

ALGAE	MOLLUSCS
Enteromorpha sp.	*Ostrea* sp.
Ulva lactuca	*Mytilus edulis aoteanus*
HYDROIDS	BARNACLES
**Pennaria* (?) sp.	**Lepas anserifera*
POLYZOA	**Balanus amphitrite denticulata*
**Schizoporella* sp.	**Balanus auricoma*
SERPULIDS	**Verruca* sp.
Hydroides norvegica	**Chthamalus hembeli*
	Balanus amphitrite cirratus
	Balanus trigonis
	**Lepas anatifera* var. a. Darwin

*Species considered to have settled on ships while in tropical waters outside the New Zealand region.

Class 6. New Zealand – Europe and Atlantic Ports

From 5 vessels of this general category the following species were collected. All vessels had dry-docked in home ports shortly before sailing for New Zealand and the few fouling organisms were taken from remote areas of the hulls.

Conchoderma virgatum, C. auritum, Balanus amphitrite denticulata, Enteromorpha sp.

Class 7. Antarctica

Damage sustained by U.S. Naval icebreakers in the course of summer activities in Antarctica resulted in 3 of these ships being docked for repair in Wellington. As would be expected from the duties carried out by these vessels, no fouling growth appeared on exposed surfaces of the hulls, but from the vicinity of sheltered ducts, many stalked barnacles *Conchoderma virgatum* and *C. auritum* were collected in each case. As the ships docked almost immediately following their arrival in New Zealand, *Conchoderma* settlement can almost certainly be regarded as having occurred during the months of Antarctic operations. Darwin (1851) observed the distribution of both species extended into polar waters of both hemispheres.

A general summary of the incidence of the major groups of fouling organisms in the various categories of shipping examined is given in Table 6.

FOULING CHARACTERISTICS OF THE MAJOR GROUPS

Algae

The chlorophyceae *Enteromorpha* and *Ulva lactuca* were the predominant fouling algae encountered and their higher incidence on ships of more southern New Zealand trade routes has been noted. *Enteromorpha* fouling was usually restricted to a narrow belt near the waterline; frequent changes

in draught characteristic of coastal cargo vessels led to desiccation of this weed with the result that prolific growth occurred only on constant draught ships.

TABLE 6—General Incidence of Major Groups of Fouling Organisms in Categories of Shipping Examined

(Total No. Ships, 89)

TRADE OR SERVICE No. Ships	LOCAL HARBOUR (32)	COASTAL (31)	AUSTRALIA (13)	PACIFIC (3)	INDIA (2)	EUROPE (5)	ANTARCTICA (3)	TOTAL RECORDS (89)
GREEN ALGAE	x	xx	x	xx	x	x	0	21
HYDROIDS	xxx	xx	xx	x	0	0	0	45
BRANCHING POLYZOA	xxx	xx	xx	0	0	0	0	55
ENCRUSTING POLYZOA	x	xx	xx	x	0	0	0	21
SERPULIDS	xxx	xx	xxx	xxx	xxx	0	0	53
STALKED BARNACLES	0	0	0	xx	x	x	xx	9
'ACORN' BARNACLES	xx	xxx	xxx	xxx	xx	xxx	0	76
MOLLUSCS	xxx	xx	xx	0	0	0	0	48
ASCIDIANS	xxx	x	x	0	0	0	0	38

0 = Absent, x = infrequent, xx = common, xxx = very common.

Ulva grew to greater depths on a ship's side (commonly up to 8–10 ft) than did *Enteromorpha.* One of the vessels examined that had been operating some months in clear tropical Pacific waters (Class 6) bore heavy *Ulva* settlements on side plates down as far as the turn of the bilges, or just over 20 ft below the waterline.

Hydroids

Because hydranths tend to be lost readily from colonies on a ship under way, this group of fouling organisms was found more abundantly on vessels listed under Class 1. As an exception to this general statement, the cosmopolitan gymnoblast *Tubularia larynx* showed considerable survival capacities and was of widespread occurrence both on internal and Australian trade routes. In the Port of Auckland this species settles continuously throughout the year, whereas in Wellington, it appears on surfaces mainly during the summer. Colonies were found with stems up to 6 in. in length on Wellington-Lyttelton express vessels and because of the clump-like growth habit such established colonies probably impose restrictions on operating efficiency as severe as any other fouling species.

Harris (1946) reported that *Tubularia* survived exposure to moderate concentrations of anti-fouling toxins, and Pyefinch and Downing were able to establish experimentally (1949) that settlement and metamorphosis of the actinula was accelerated in the presence of copper ions. While most *Tubularia* colonies seen were attached to paint surfaces, the majority of other hydroids appeared as epizoites upon barnacles, tubeworms, and encrusting polyzoa.

Serpulids

Considering all categories of shipping examined, *Hydroides norvegica* ranked as the most ubiquitous fouling species. On one route (New Zealand – India) it alone gave rise to severe fouling of both vessels inspected. It is known as a fouling organism of world-wide distribution in warmer seas (Woods Hole Oceanographic Institution 1952) and has been recorded locally as a summer settling form from Auckland to at least as far south as Lyttelton (Skerman, 1958b, 1959). Rapid growth of the calcareous tubes, up to 1 in. per month, was characteristic of the warmer part of this range. Settlement in high concentrations, as shown in Fig. 2 was marked by the ends of tubes growing away from the hull surface, a tendency which may be expected to contribute greatly increased frictional resistance to ship movement. A point of further interest in this illustration is the vertical orientation of the tube apertures towards the direction of incident light.

Galeolaria hystrix achieved greatest abundance on shipping associated with southern New Zealand waters, but settlement was never observed in dense formations as with *Hydroides*. In crowded assemblies of other organisms growth is directed away from the substratum and, after extended out-of-dock periods, tubes up to 4 in. in length were often collected.

Pomatoceros carciniferus was an infrequent fouling serpulid, and is locally regarded as of purely intertidal distribution (G. A. Knox, pers. comm.).

Polyzoa

The cosmopolitan species *Bugula neritina* was the most widely occurring polyzoan on coastal and Australian routes, and settlement was particularly heavy on vessels laid up in Auckland. *B. flabellata* was more prominent on coastal routes south of Auckland, as was *Cryptosula pallasiana,* the commonest encrusting polyzoan appearing on coastal shipping. *Cryptosula* has been recorded from South Australia (Brown 1952) and is widely known as a fouling species in the northern hemisphere (Scheer, 1945). It is capable of rapid growth spreading over the shells of barnacles and serpulids to occupy areas up to 3–4 sq. in. *Bugula avicularia,* like *Caberea zelandica,* was found chiefly on ships operating in the Auckland region and it also appeared sporadically on Trans-Tasman vessels.

Pyefinch (1950) observed that polyzoa seldom survived sea journeys on the vessels he examined, but colonies of the common species recorded here were usually found intact. No doubt the journeys involved in the local ships examined were of shorter duration than was the case with Pyefinch's examples.

Molluscs

Mollusc fouling was conspicuous only on internal trade routes and shipping confined to the ports of Auckland and Wellington. Mussels were the main forms encountered south of the Auckland region in which oysters predominated, but the development of both groups as important fouling organisms was usually indicative of out-of-dock periods of about 12 months or more. Settlement of *Mytilus edulis aoteanus* occurred on all underwater plating of vessels immobilised for lengthy periods in Wellington Harbour, while the larger *Perna canaliculus* was more frequently confined to lower plates and keel regions. Although none of the Auckland vessels examined displayed much mussel fouling, the author has been informed that vessels laid up in Auckland Harbour for some years acquire heavy mussel growth which is predominantly *Perna canaliculus*.

Difficulties in identifying fouling oysters have been discussed previously (Skerman, 1959, p. 71). Tentative determinations only have been attempted for material in the present survey and most appear to possess characteristics resembling *Ostrea sinuata* Lamarck rather than *Crassostrea glomerata,* a species known chiefly from coasts of the North Auckland peninsula where it occupies higher levels of the inter-tidal zone. It is possible that ship-fouling oysters from the Auckland region may ultimately be proved as *Crassostrea,* having undergone some alterations in growth form simulating *Ostrea* under the unnatural conditions of permanent submergence upon a ship's hull. Wood and Allen (1958) list the common fouling oyster in Australian ports as *Crassostrea commercialis*.

Barnacles

The occurrence of *Elminius modestus* on nearly 70% of shipping in New Zealand waters (Classes 1 and 2) may be attributed to its capacity for reproduction and settlement over the whole year throughout the country and its tolerance of a wide range of salinities and sea temperatures. On routes south of Auckland it appeared as the sole fouling barnacle of importance. Settlement occurred on all underwater areas of a hull, but denser populations were commonly found upon plate landings, small recesses and on the outer edge of the bilge keels, a tendency which possibly reflects influences of illumination and surface features upon larval behaviour at the time of initial settlement.

The ability of some fouling barnacles to undercut paint films during growth of the basal edges of the shell compartments has not been observed in *Elminius,* possibly because of the more delicate membranous basis it possesses. As a result, *Elminius* populations are relatively easily dislodged from a hull in dock by the usual scraping methods. The spread of *Elminius modestus* from southern Australasia to northern European waters that has been recognised in the last 20 years was originally attributed to its presence on ships travelling between these two regions (Bishop, 1947). In this survey it could not be established with certainty whether the settlements found on the few overseas vessels examined had been carried on round voyages or not. Neither of the other two *Elminius* species of New Zealand coasts (Moore 1944), *E. simplex* and *E. plicatus,* appeared as fouling organisms: the latter species is known as an intertidal form.

In Auckland Harbour, *Balanus amphitrite,* like *Elminius,* was capable of a high rate of settlement from November to April. Pope (1945) noted that in the vicinity of Sydney Harbour, *B. amphitrite cirratus* shares with *Elminius* a tolerance of a wide range of salinities, also Skerman (1959) found a higher incidence of both species in the more estuarine upper reaches of Waitemata Harbour. The salinity factor may have influenced the conspicuously heavy settlement by these barnacles found on shipping associated with Onehunga on the Manakau Harbour in which Wallace *et al.* (1956) found salinities varying from 18·7‰ to 35·8‰.

Though *B. amphitrite communis* appeared commonly on coastal ships calling at Auckland, its incidence was lower than of *cirratus,* and it seems to have been even of less relative importance on Trans-Tasman vessels.

Balanus trigonis made its most frequent appearance as a fouling barnacle on naval ships berthed at Devonport (Class 1). Test panels in this vicinity (Station A4, Skerman 1959) also displayed higher *B. trigonis* settlement than at other sites within the harbour.

In a report on the seasonal occurrence of fouling organisms in the Port of Auckland (Skerman 1959) it was observed that many shells of *Balanus amphitrite cirratus* attached to test panels were occupied by turbellarian flatworms, and this was also found to be a common feature of barnacles taken from ships of the trans-Tasman service, R.N.Z.N. ships at Auckland and a number of coastal vessels calling at Auckland. *B. a. cirratus* and less frequently *B. trigonis* were again the species primarily affected. The polyclad has since been identified as *Stylochus zanzibaricus* Laidlaw and is the subject of a separate communication (Skerman 1960).

Heavy mortalities in *Balanus* populations (*B. amphitrite* and *B. trigonis*) were notable on ships from Auckland and Australia that entered southern New Zealand ports in winter months. Although south of the natural range of both species, populations appeared to survive such journeys at other times of the year.

Barnacle Growth on Anti-fouling Paints

Many specimens of *Balanus trigonis* taken from vessels laid up at Auckland were found to have unusually distorted shells although they did not appear to have been growing under particularly crowded conditions. Weiss (1948) noted shell deformation to be a common feature of barnacles that had grown on paints containing copper toxins, and Stubbings (1959) has made similar observations with regard to *B. amphitrite stutsburi.* All vessels from which distorted *B. trigonis* were collected had been applied with anti-fouling paints, but no analyses have been made to determine the initial or residual copper content likely to have affected barnacle growth. Figure 3 illustrates a typical example of shell malformation in *B. trigonis,* showing the deeply incised periphery of the thickened concave basis, and laterally, the strong deformation of the compartments. A normal specimen from a non-toxic test panel is shown for comparison. No specimen of the var. *cirratus* of *B. amphitrite* from the same source exhibited this type of shell deformation.

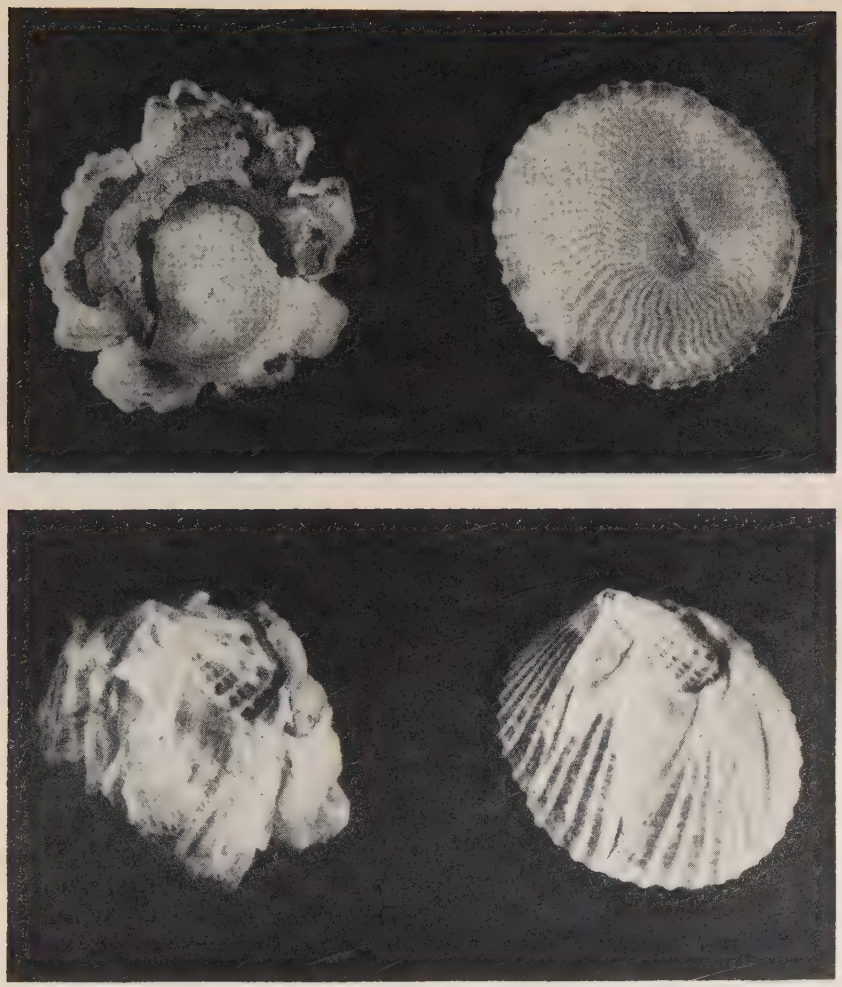

Photo: H. D. O'Kane

FIG. 3—Shell deformation in *Balanus trigonis* removed from a ship's hull painted with an anti-fouling composition. Upper left shows the distorted concave basis, lower left the deformed lateral compartments. A normal specimen from a non-toxic test panel is shown for comparison on the right of each illustration. × 4.

SEQUENCES OF SETTLEMENT ON TOXIC PAINT SURFACES

In fouling developing on many naval reserve vessels at Auckland (Class 1), there appeared a characteristic pattern in the settlement of the predominating barnacles, polyzoa, and ascidians. Barnacles were the main macroscopic group attached directly to the paint surfaces while the majority of the abundant *Bugula neritina* colonies had their holdfast structures upon

the barnacle shells. Growth of the compound ascidian *Botrylloides leachi* was similarly restricted to the bases of polyzoan colonies and barnacle crests, and seldom grew on the paint surface. This sequence in settlement may well have arisen under the influence of anti-fouling paints, initial establishment of the barnacle populations providing a non-toxic substratum for growth of the polyzoan and ascidian elements that may have been inherently more susceptible to the toxic compounds used. Examples of successive stages in this pattern of settlement are shown in Fig. 4; all could

FIG. 4—Sequences of settlement on toxic surfaces. Left, a barnacle, *Balanus trigonis* removed from an anti-fouling paint surface. Middle, epizoic growth of a polyzoan colony, *Bugula neritina,* and a colonial ascidian, *Botylloides leachi* upon the shells of barnacles. Right, ultimate growth of *Botylloides* enveloping both barnacles and polyzoan.

be found on the one ship, but the more advanced the degree of fouling (or time out of dock) the higher the proportion of ascidian overgrowth that appeared.

BARNACLE SURVIVAL ON MOVING SURFACES

In examining the effect of water currents over a surface on barnacle settlement, survival and growth, Smith (1946) found a velocity of about 1 knot appeared to be the maximum rate permitting attachment. Slight variations were noted between the three species used in his tests. Following attachment, growth was found to be increased in water currents in excess of this rate, but once attached, the barnacles were able to withstand current velocities

of increasing magnitude, and ultimate loss of attachment appeared to follow reduced growth rate and may have involved death from an inability to feed.

In view of these experimental data, the settlement of *Elminius* upon the propeller boss and blades shown in Fig. 5 is of interest. The vessel concerned

FIG. 5—Distribution of surviving *Elminius modestus* barnacles on a propeller boss and blades of a coastal vessel engaged in a regular (daily) passenger service.

was a Wellington–Lyttelton passenger steamer and docking followed soon after an 11 month period of continuous service. Barnacle settlement was uniform over the surface of the boss and extended about 6 in. outwards on the trailing edges of the blades to a maximum distance of 1 ft 9 in. from the axis of the shaft. All specimens were alive at the time of inspection in dock. With screws turning at 180 r.p.m. at normal cruising speed of the vessel, the nominal velocity of water currents relative to the blades at the circumference of the barnacle-fouled area would be 19·5 knots. The *actual* velocity of water currents immediately by the barnacles, however, may be somewhat less than this figure because of frictional drag and vorticellar flow, yet in this calculation, no account has been taken of the ship's own speed of 18–20 knots at these revolutions, and the approximation may therefore be even a considerable underestimation of the water currents and sheer forces withstood by the barnacles. Probably the 13 hours spent daily in harbour (36 hours at the week end) between journeys of 11 hours' duration were sufficient to allow feeding and thereby sustain growth of the attached barnacles. No similar case of barnacle fouling of propellers was observed on ships employed on longer journeys.

CORROSION

Plates of a number of coastal vessels examined displayed evidence of widespread corrosion beneath paint films that superficially appeared intact. This was particularly noticeable when docking took place in warm weather when droplets of dark fluid could be seen exuding from pinholes or hairline cracks in the paint surface on plates exposed to the sun (Fig. 6). Fracturing and removal of the paint layers in these areas invariably revealed complete loss of adhesion with the underlying metal which was bright and frequently deeply pitted. A black sludge-like deposit which was found to consist mainly of iron sulphide lay between the metal and the hard crust comprised of successive layers of paint.

Many investigators have studied the anaerobic corrosion of iron and steel in the marine environment. The early work indicated that sulphate-reducing bacteria (*Desulphovibrio* spp.) were implicated in the process, it being generally assumed that hydrogenase-containing strains bring about the cathodic depolarisation of the iron. More recently (e.g. Starkey 1953), it has been proposed that the principal contribution of sulphate-reducers to the corrosion process is the formation of hydrogen sulphide; this, and perhaps other sulphur compounds accumulating from other forms of microbial metabolism, may act either directly, or indirectly, in the formation of corrosion cells with the metal. In the present example there must be considered, in addition to bacterial action, the likelihood of corrosion cells between the copper or compounds of copper in overlying anti-fouling paints and the steel that could arise following the breakdown of intervening priming and "anti-corrosive" systems.

No details of these corrosion processes were pursued, but it seems that the conditions observed on these vessels resulted from the practice of applying successive coats of paints upon systems of which the primary adhesion to steel had already deteriorated. Entry of sea water through pinholes in the paint

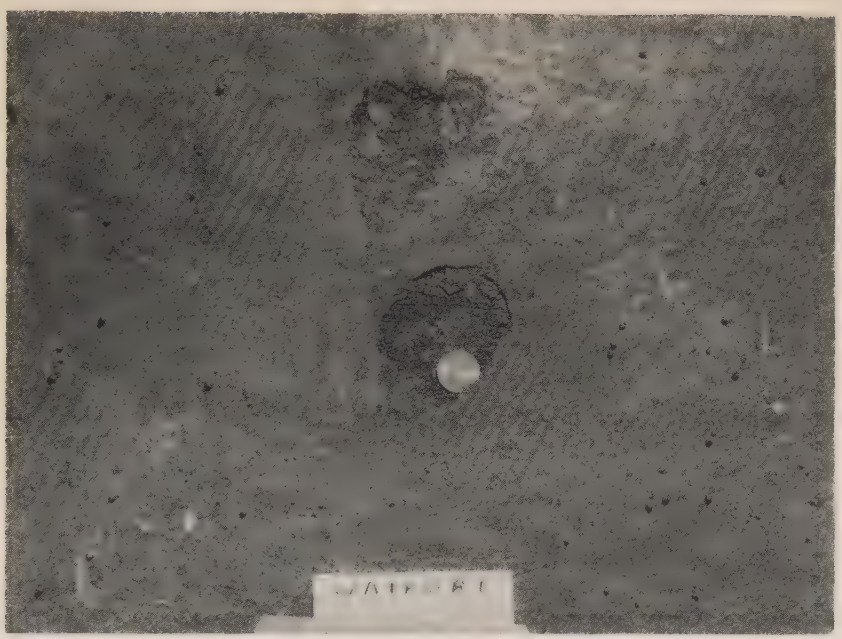

FIG. 6—Illustration of an example of the corrosion of steel plates beneath the paint film. In the centre a small area of paint crust has been removed to show black corrosion products overlying the steel, while on each side of this are droplets of dark fluid which has seeped out from beneath the poorly adhering paint film.

film would, in addition to providing a conducting medium, ultimately give rise to an environment low in oxygen tension that would favour bacterial sulphate-reduction.

The incidence of this form of corrosion on coastal shipping suggests that some alteration in local docking practice may be justified wherein hull plates are stripped of old paint, sandblasted and reprimed on a rotation basis every few years.

DISTRIBUTION OF FOULING SPECIES ON HULL SURFACES

Apart from the well known bands of green seaweed fouling in the vicinity of waterline plates, and the tendency for heavier growth to accumulate on bottom areas shielded from direct light, few generalisations on the distribution of animal fouling on hull surfaces can be made concerning vessels in service. Concentrations of barnacles, particularly *Elminius* by plate landings, sea-water intake vents and the edges of bilge keels have already been referred to. In mussel fouling, the smaller form, *Mytilus*, usually appeared nearer the waterline than did *Perna* populations which were generally found on bottom plates. Hydroids, especially *Tubularia* characteristically attached to poorly illuminated areas below the bilge keels and under the stern counter, while polyzoa (*Bugula* spp.) were more frequently recorded towards the bows and at higher levels of the ships' sides.

Irregular distributions of fouling growths in several instances could be attributed to inconsistencies in previous painting techniques. Figure 7 shows a vertical band of *Elminius* immediately below deck wash ports on the side

FIG. 7—Heavy barnacle settlement in a zone of a ship's side beneath discharge ports, seepage from which had prevented paint adhesion during the previous dry docking

of a coastal ship in dry dock; apparently water discharging through the port and down the ship's side during application of the anti-fouling paint in this case had prevented effective paint adhesion, so creating a non-toxic area. In early stages of the survey it was a common docking practice to apply "anti-galvo" compositions to rudder and stern plates bordering the propellers, and such non-toxic surfaces too became susceptible to heavy settlement. This practice has since been discontinued. Draught marks, painted after application of anti-fouling compositions similarly are prone to early settlement by fouling growths.

DISCUSSION

It is stressed that the tabulated data on the frequency of occurrence of the species recorded are not necessarily a true indication of their relative importance as fouling organisms. This latter consideration involves the capacity of each form to offer impedence of a ship's movement through the water and is a function not only of its abundance, but also of its particular growth habit. Also it is obvious that some of the commonest species recorded (e.g. *Elminius*) are those which breed and settle over a long period of the year; comparing their frequency on ships with that of species of restricted seasonal occurrence may tend to minimise the importance of forms observed less often but of severe fouling potential. Again, the presence of other species, such as the encrusting polyzoa *Cryptosula* and *Watersipora,* may not contribute much frictional resistance to ship movement, but becomes of indirect importance in providing a non-toxic substratum for the settlement of less desirable fouling species inherently more susceptible to anti-fouling toxins.

Apart from the small variations noted as peculiar to individual cases, there is an overall homogeneity in the qualitative nature of fouling arising on internal trade routes within a zone south of the southern half of the North Island. Subtropical conditions north of this zone are marked by the appearance of species such as *Balanus amphitrite cirratus* as fouling components which distinguish vessels that include Auckland as a port of call between October and April. Presumably such distinguishing features of fouling in the Auckland region would be less evident outside the settlement season of the relevant species during the winter, but out-of-dock periods of the order of twelve months in the examples surveyed here intercepted settling seasons of all species.

With only an arbitrary basis of comparison, and many different variables between a relatively small number of sampled vessels, differences in fouling intensity between trade routes cannot be defined clearly. From the observations made, the impression was gained that for equal out-of-dock periods, fouling on internal routes was more severe on vessels that included northern North Island ports of call in their itinerary than on those confined to more southern routes. Even heavier fouling growth appeared on some ships operating on trans-Tasman lanes. Of individual New Zealand ports, Auckland would no doubt rank highest in fouling intensity as shown by the abundance and rapid growth particularly of the barnacles, polyzoa, and *Tubularia* appearing on naval vessels in reserve. From these records, together with those of test

panels, it is evident that any ship lying idle in the Port of Auckland would be exposed to a high rate of settlement in all but mid-winter months of the year; some of the factors contributing to heavy marine fouling within Auckland Harbour have been discussed previously (Skerman, 1959). Most of the above generalisations are well known to the local shipping trade.

The question of fouling species from overseas ports being recently introduced to New Zealand waters is a difficult matter to resolve in a country where many elements of the littoral and shallow water marine communities are only just becoming recognised. The cosmopolitan distribution of species such as *Bugula neritina, B. avicularia, Botryllus schlosseri, Enteromorpha intestinalis* is commonly regarded as a result of their transposition by ships, but seldom can the initial establishment of a species within a region previously foreign to its range be traced. The barnacle, *Balanus amphitrite cirratus* had not been identified as such in New Zealand waters prior to the author's observations in Auckland Harbour in 1954–56 (Skerman, 1959). A common sub-tidal barnacle of East Australian coasts and estuaries (Pope, 1945), this species may well have spread to Auckland waters by the agency of trans-Tasman shipping, but information on such a possibility in the absence of previous faunal records can only be inconclusive. These considerations emphasise the need for basic biological studies of marine fouling organisms, particularly with regard to their distribution, reproductive cycles, physiology and settling behaviour; and as the fouling characteristics in regions of the world important to the shipping trade become more widely understood, the better will technology be equipped in dealing with the problems engendered.

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THE COVARIANCE ANALYSIS OF TIME-REPLICATED DATA

By P. WHITTLE* and H. S. ROBERTS, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, Wellington

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Summary

The analysis of covariance is discussed where the regression coefficient varies with time.

INTRODUCTION

The analysis of covariance is widely used as a means of eliminating extraneous variation in an experiment. Thus, suppose one is interested in the final weights y_i of a number of animals which have each undergone a different treatment, y_i being the weight of the i th animal which has been given the i th treatment. The magnitude of y_i will be determined partly by the treatment and partly by the animal's own intrinsic weight of which the *initial weight* x_i is in some degree a measure. Since it is the effect of treatment in which one is interested, instead of analysing the raw weights, one analyses the *corrected weights*

$$y_i - \alpha x_i \tag{1}$$

in which the effect of initial weight has been eliminated as well as it can be by a linear correction. Here α is a suitably determined regression coefficient. This correction modifies the variance analysis, so that instead of considering the raw sum of squares

$$S = \Sigma (y_i - y_\cdot)^2$$

one considers a corrected sum

$$S^* = \Sigma (y_i - y_\cdot)^2 - \frac{[\Sigma (y_i - y_\cdot)(x_i - x_\cdot)]^2}{\Sigma (x_i - x_\cdot)^2} \tag{2}$$

The technique extends readily to more complicated situations. Thus, suppose one has a double classification y_{ij}, and is considering deviations of the type

$$y'_{ij} = y_{ij} - y_{i\cdot} - y_{\cdot j} + y_{\cdot\cdot} \tag{3}$$

*Now at Cambridge University.

instead of using a raw sum of squares

$$S = \Sigma\Sigma(y'_{ij})^2 \tag{4}$$

one uses

$$S^* = \Sigma\Sigma(y'_{ij})^2 - \frac{[\Sigma\Sigma y'_{ij}x'_{ij}]^2}{\Sigma\Sigma(x'_{ij})^2} \tag{5}$$

where x'_{ij} is constructed in the same way as y'_{ij}.

In this paper we shall consider the modified situation in which repeated measurements are made on the animal in question, so that instead of a set of yields y_{ij} one has a set y_{ijk}, the subscript k covering the measurements taken at different times on the same animal. This situation is obviously a common one, and arises in experiments on different sorts of material. Thus, in investigating the effects of treatments on fruit trees or perennial crops it is only natural to repeat measurements over a number of years, and the same holds in the investigation of long-term effects of fertilisers on soils. One will probably have recourse to the analysis of covariance in both cases, to correct for initial differences in trees and in soil plots respectively.

However, it is apparent that from the point of view of analysis such a situation differs from the one in which time replication did not enter. Since the dependence of a measurement y_{ijk} on the initial value x_{ij} will almost certainly vary with time one will have to consider corrected variates,

$$y_{ijk} - \alpha_k x_{ij} \tag{6}$$

for which the regression coefficient α_k varies with time. Furthermore, if we are considering sums of squares corresponding to deviates such as

$$y'_{ijk} = y_{ijk} - y_{\cdot jk} - y_{i\cdot k} - y_{ij\cdot} + y_{i\cdot\cdot} + y_{\cdot j\cdot} + y_{\cdot\cdot k} - y_{\cdot\cdot\cdot} \tag{7}$$

it is not immediately obvious what form the corrected sum (5) will take. This is because x_{ij} does not depend upon k, and so one cannot construct an x'_{ijk} which is analogous to the y'_{ijk} of (7).

The rule which emerges from the analysis of this paper is simple: expression (5) still holds for the different sums of squares if the x deviates are built up in the same way as the y deviates, except that all eliminations of year effects are omitted. Thus if

$$y'_{ijk} = y_{ijk} - y_{i\cdot k} \tag{8}$$

then

$$x'_{ij} = x_{ij} - x_{i\cdot} \tag{9}$$

but if y'_{ijk} is given by (7) then

$$x'_{ij} = x_{ij} - x_{i\cdot} - x_{\cdot j} + x_{\cdot\cdot} \tag{10}$$

The presentation of the analysis of covariance in almost all the standard textbooks is rather superficial and unconvincing, abounding in phrases of the

type "the sum of squares may obviously be split into . . . ". We shall take the opportunity in this paper to set out explicitly the model and assumptions on which the technique is based.

NOTATION

We shall use the dot notation for sample means so that $y_{\cdot jk}$ is the sample mean of y_{ijk} for varying i and fixed j, k; $y_{\cdot\cdot k}$ is the sample mean for varying i, j and fixed k, etc. We also adopt the notation

$$y_{ijk}{}^{i} = y_{ijk} - y_{\cdot jk} \tag{11}$$

$$y_{ijk}{}^{ij} = y_{ijk} - y_{\cdot jk} - y_{i\cdot k} + y_{\cdot\cdot k} \tag{12}$$

$$y_{ijk}{}^{ijk} = y_{ijk} - y_{\cdot jk} - y_{i\cdot k} - y_{ij\cdot} + y_{i\cdot\cdot} + y_{\cdot j\cdot} + y_{\cdot\cdot k} - y_{\cdot\cdot\cdot} \tag{13}$$

the principle being that the superscripts refer to effects which have been eliminated in the deviate, i.e. all subscripts which appear in the first term of the right-hand member, but not in the last term. Correspondingly:

$$x_{ij}{}^{j} = x_{ij} - x_{i\cdot} \tag{14}$$

$$y_{i\cdot k}{}^{ik} = y_{i\cdot k} - y_{\cdot\cdot k} - y_{i\cdot\cdot} + y_{\cdot\cdot\cdot} \tag{15}$$

Finally, since so many of the sums of squares fall into the standard form (5) we shall define a quantity

$$S(x^a, y^b) = \Sigma (y^b)^2 - \frac{(\Sigma x^a y^b)^2}{\Sigma (x^a)^2} \tag{16}$$

Here a and b are two arbitrary sets of superscripts, and the summation Σ covers all subscripts.

THE MODEL AND THE ESTIMATION OF CONSTANTS

The experiment which motivated this investigation was one concerning apple trees (Tiller *et al.* 1959). The trees were laid out in randomised blocks and different treatments were apportioned. The tree yields were measured every year so that we have a series of observations y_{ijk}, where i, j and k indicate block, treatment, and year respectively. Before the treatments were applied however, the trees were run for a time in a uniformity trial, so that one has a series of *initial yields* x_{ij} which enable one to eliminate individual tree variation to some degree.

We shall restrict ourselves to consideration of this particular design which is sufficiently general to make the course of further generalisation in several directions fairly evident.

We suppose that the data may be represented by the linear model

$$y_{ijk} = m + b_i + t_j + z_k + c_{ij} + d_{jk} + f_{ik} + \alpha_k u_i + \beta_k v_j + \gamma_k w_{ij} + \epsilon_{ijk} \tag{17}$$

where

$$\begin{aligned} u_i &= x_{i.}{}^{i} = x_{i.} - x_{..} \\ v_j &= x_{.j}{}^{j} = x_{.j} - x_{..} \\ w_{ij} &= x_{ij}{}^{ij} = x_{ij} - x_{i.} - x_{.j} + x_{..} \end{aligned} \tag{18}$$

The constants b, t, z, c, d, f are the usual main effect and interaction constants, whose estimation is rendered determinate by application of the conditions

$$\sum_i b_i = \sum_j t_j = \sum_k z_k = \sum_i c_{ij} = \sum_j c_{ij} = \sum_j d_{jk} = \sum_k d_{jk} = \sum_i f_{ik} = \sum_k f_{ik} = 0 \tag{19}$$

The constants α_k, β_k and γ_k are the regression coefficients for block, treatment and residual effects respectively. If one were convinced that these different effects all depended on initial conditions in the same way i.e. that

$$\alpha_k = \beta_k = \gamma_k \tag{20}$$

then it would be sufficient to include a single regression term $\alpha_k x_{ij}$ in the representation. On the other hand, if one felt that allowance should be made for the possible variation of strength of regression between different blocks and/or different treatments then it would be necessary to modify the regression terms to

$$\alpha_{jk} u_i + \beta_{ik} v_j + \gamma_k w_{ij} \tag{21}$$

We shall restrict attention to model (17) however, which is sufficiently flexible for most purposes.

The constants must be further restricted before the model is determinate. For instance, it is obvious that the terms $t_j + d_{jk}$ and $\beta_k v_j$ "overlap" to some extent, i.e. that a change in one can be partially compensated for by a change in the other. In other words, some of the variation over treatment groups is genuinely due to treatments and some of it due to initial differences, v_j in treatment group means, but there is no simple way of correctly assigning the variation between these two sources. One is led to the rather arbitrary step of setting up orthogonality conditions

$$\sum_j v_j t_j = \sum_j v_j d_{jk} \tag{22}$$

in order to ensure that the two sets of terms shall have no "common ground". One is in effect letting the initial differences v_j explain as much variation as possible, so that the residual variation is orthogonal to the v_j sequence This step alters the interpretation of the constants: t_j is now not an unbiased measure of the treatment effects, but is a measure of that part of the treatment effect which could not possibly be attributed to initial variation in treatment group means.

Indeterminancies in the block and interaction constants are removed similarly by application of the conditions

$$\sum_i b_i u_i = \sum_i f_{ik} u_i = \sum_i \sum_j c_{ij} w_{ij} = 0 \tag{23}$$

Minimisation of the residual sums of squares subject to conditions (19), (22) and (23) leads to the normal equations

$$\begin{aligned}
&y_{\ldots} = m \\
&y_{i..} = m + b_i + \alpha . u_i \\
&y_{.j.} = m + t_j + \beta . v_j \\
&y_{..k} = m + z_k \\
&y_{.jk} = m + t_j + z_k + d_{jk} + \beta_k v_j \\
&y_{i.k} = m + b_i + z_k + f_{ik} + \alpha_k u_i \\
&y_{ij.} = m + b_i + t_j + c_{ij} + \alpha . u_i + \beta . v_j + \gamma . w_{ij} \\
&\sum_i u_i (y_{i.k} - \alpha_k u_i) = 0 \\
&\sum_j v_j (y_{.jk} - \beta_k v_j) = 0 \\
&\sum_i \sum_j w_{ij} (y_{ijk} - \gamma_k w_{ij}) = 0
\end{aligned} \tag{24}$$

which have the solution

$$\begin{aligned}
&b_i = y_{i..}{}^{i} - \alpha . u_i \\
&t_j = y_{.j.}{}^{j} - \beta . v_j \\
&z_k = y_{..k}{}^{k} \\
&c_{ij} = y_{ij.}{}^{ij} - \gamma . w_{ij} \\
&d_{jk} = y_{.jk}{}^{jk} - (\beta_k - \beta .) v_j \\
&f_{ik} = y_{i.k}{}^{ik} - (\alpha_k - \alpha .) u_i
\end{aligned} \tag{25}$$

$$\alpha_k = \frac{\sum u_i y_{i.k}{}^{i.k}}{\sum u_i^2} = \frac{\sum u_i y_{i.k}}{\sum u_i^2}$$

$$\beta_k = \frac{\sum v_j y_{.jk}{}^{.jk}}{\sum v_j^2} = \frac{\sum v_j y_{.jk}}{\sum v_j^2}$$

$$\gamma_k = \frac{\sum\sum w_{ij} y_{ijk}{}^{ijk}}{\sum\sum w_{ij}^2} = \frac{\sum\sum w_{ij} y_{ijk}}{\sum\sum w_{ij}^2}$$

In view of the fact that the minimisation is conditioned by (19), (22) and (23) one should strictly have added these expressions (each one multiplied by its own Lagrangian multiplier) to the sum of squares being minimised. However, it is easy to show by manipulation of the resulting set of normal equations that all the Lagrangian multipliers are zero, and so need not be considered. In the next section we shall calculate the sum of squares for the different effects by leaving the corresponding constants out of the representation (17) and calculating the increase in the residual sum of squares. In this case (i.e. when some of the constants have been omitted) not all the Lagrangian multipliers are zero; in fact the multipliers take the place of the omitted constants and assume what would have been their estimated values. This means that estimates of remaining constants are not changed, i.e. the model is orthogonal.

SUM OF SQUARES

Inserting the estimated values of the constants (25) in the representation (17) we find that the estimated residual may be expressed

$$\epsilon_{ijk} = y_{ijk}{}^{ijk} - (\gamma_k - \gamma_\bullet)w_{ij} \tag{26}$$

so that the residual sum of the squares is

$$\Sigma\Sigma\Sigma\, \epsilon_{ijk}{}^2 = \mathrm{S}\,(x_{ij}{}^{ij}\,, y_{ijk}{}^{ijk}) \tag{27}$$

Let us now distinguish all parameter estimates (25) by a bar (i.e. $\bar{b}_i$ $\bar{\alpha}_k$, $\bar{\varepsilon}_{ijk}$).

Consider now the sum of squares due to blocks. Omission of block constants from (17) leads to no change in remaining parameter estimates thanks to the orthogonality of the model, so that the new residual is related to the old by

$$\epsilon_{ijk} = \bar{\epsilon}_{ijk} + b_i \tag{28}$$

The sum of squares due to blocks is thus

$$\Sigma\Sigma\Sigma(\epsilon_{ijk}{}^2 - \bar{\epsilon}_{ijk}{}^2) = \Sigma\Sigma\Sigma b_i{}^2 = \mathrm{S}(x_{i\cdot}{}^i\,, y_{i\cdot\cdot}{}^i) \tag{29}$$

by (25).

The remaining sums of squares for the factors of the experiment can be calculated similarly, the results are summarised in the following table:

EFFECT	SUM OF SQUARES	DEGREES OF FREEDOM
Blocks	$S(x_{i\cdot}{}^{i}, y_{i\cdot\cdot}{}^{i})$	$p-2$
Treatments	$S(x_{\cdot j}{}^{j}, y_{\cdot j\cdot}{}^{j})$	$q-2$
Years	$S(x_{\cdot\cdot}, y_{\cdot\cdot k}{}^{k})$	$r-1$
Blocks × Treatments	$S(x_{ij}{}^{ij}, y_{ij\cdot}{}^{ij})$	$(p-1)(q-1)-1$
Treatments × years	$S(x_{\cdot j}{}^{j}, y_{\cdot jk}{}^{jk})$	$(q-1)(r-1)-r$ (30)
Blocks × years	$S(x_{i\cdot}{}^{i}, y_{i\cdot k}{}^{ik})$	$(r-1)(p-1)-r$
Residual	$S(x_{ij}{}^{ij}, y_{ijk}{}^{ijk})$	$(p-1)(q-1)(r-1)-r+3$

We have assumed that there are p blocks, q treatments and r years. The sums of squares are evidently a very simple generalisation of those for the unreplicated design: the x deviates are constructed in the same way as the y deviates except that the k indices are omitted.

The degrees of freedom are arrived at by counting the number of parameters which have been estimated for the effect under consideration and then subtracting the number of constraints to which these same parameters are subject.

The degrees of freedom for the table add up to $pqr-3r-1$, the deficit of $3r$ being due to the $3r$ regression coefficients which have been estimated. The sum of squares for the regression effect is best considered separately, because it can be broken up in so many ways. This profusion of possible hypotheses is one of the characteristic features of a full covariance analysis.

The calculation of sums of squares is based on the following fact, which is readily verified: that if the introduction of a restricting hypothesis modifies the α_k estimate from $\bar{\alpha}_k$ to $\hat{\alpha}_k$ etc., then the sum of squares due to the lost degrees of freedom is

$$\Sigma\Sigma\Sigma\,[\,(\hat{\alpha}_k-\bar{\alpha}_k)^2u_i^2+(\hat{\beta}_k-\bar{\beta}_k)^2v_j^2+(\hat{\gamma}_k-\bar{\gamma}_k)^2w_{ij}{}^2\,] \tag{31}$$

Thus, if we wish to test the hypothesis that $\alpha_k=0$ then we have $\hat{\alpha}_k=0$, the remaining regression coefficients are unaffected and the sum of squares due to block regressions is

$$\Sigma\Sigma\Sigma(\bar{\alpha}_k u_i)^2=q(\underset{k}{\Sigma}\bar{\alpha}_k{}^2)\,(\underset{i}{\Sigma}u_i{}^2)=q\underset{k}{\Sigma}\frac{(\underset{i}{\Sigma}u_i\,y_{i\cdot k}{}^{i})^2}{\underset{i}{\Sigma}u_i{}^2}\qquad(\text{D.F.}=r) \tag{32}$$

This sum of squares may be boken down into a component for the time variation of the α's and one for the departure of the mean α . from zero if we consider the two successive hypotheses

$$\begin{aligned} \alpha_k &= \alpha \\ \alpha &= 0 \end{aligned} \tag{33}$$

We find thus that the sum of squares due to the departure of the mean block regression from zero is

$$q r \bar{\alpha}.^2 (\Sigma u_i^2) = q \sum_k \frac{(\sum_i u_i y_{i\cdot\cdot}^{\,i})^2}{\sum_i u_i^2} \qquad \text{(D.F.} = 1) \tag{34}$$

while the sum of squares due to variation of block regressions is

$$q \sum_k (\bar{\alpha}_k - \bar{\alpha}.)^2 \, (\sum_i u_i^2) = q \sum_k \frac{(\sum_i u_i \, y_{i\cdot k}^{\,ik})^2}{\sum_i u_i^2} \tag{35}$$

The formulae for treatment and block $\times$ treatment interaction regressions follow similarly.

Another set of natural hypotheses to test is whether any or all of the three sets of regressions α_k, β_k, γ_k are equal. To test the hypothesis

$$\alpha_k = \beta_k \tag{36}$$

we use (31) with r degrees of freedom, substituting

$$\hat{\alpha}_k = \hat{\beta}_k = \frac{q \sum_i u_i y_{i\cdot k} + p \sum_j v_j y_{\cdot jk}}{q \sum_i u_i^2 + p \sum_j v_j^2} \tag{37}$$

$$\hat{\gamma}_k = \bar{\gamma}_k$$

To test the hypothesis

$$\alpha_k = \gamma_k \tag{38}$$

we use (31) (D.F. = r) with

$$\hat{\alpha}_k = \hat{\gamma}_k = \frac{q\sum_i u_i y_{i\cdot k} + p\sum\sum_{i\,j} w_{ij} y_{ijk}}{q\sum_i u_i^2 + \sum\sum_{i\,j} w_{ij}^2} \tag{39}$$

$$\hat{\beta}_k = \bar{\beta}_k$$

The test of the hypothesis $\beta_k = \gamma_k$ is analogous. To test the hypothesis

$$\alpha_k = \beta_k = \gamma_k \tag{40}$$

we use (31) (D.F. = 2r) with

$$\hat{\alpha}_k = \hat{\beta}_k = \hat{\gamma}_k = \frac{\sum\sum (x_{ij} - x_{\cdot\cdot}) y_{ijk}}{\sum\sum (x_{ij} - x_{\cdot\cdot})^2} \tag{41}$$

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MINERALOGY AND RADIOACTIVITY OF NIUE ISLAND SOILS

By M. Fieldes, G. Bealing, G. G. Claridge, N. Wells, N. H. Taylor,
Soil Bureau, Department of Scientific and Industrial Research,
Wellington

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Summary

Results of X-ray, D.T.A., magnetic, and radioactivity examinations of Niue Island soils (from samples collected in 1949) are given and problems of soil origin and agriculture are discussed.

The mineralogy of the soils is unusual and they show considerable natural radioactivity. These soils have silicon contents of less than 1% and average phosphorus content of about 1%.

Hakupu soils of the elevated coastal fringe contain up to 40% of crandallite ($Ca\ Al_3\ (PO_4)_2(OH_6)$) 2% to 5% magnetite, much goethite, and some gibbsite. Fonuakula and Palai soil types of the inner basin consist mainly of gibbsite and goethite with up to 8% crandallite and 1% magnetite. Crandallite decreases and gibbsite increases in passing from the raised fringe to the central depression. Crandallite contents are limited by contents of phosphorus.

The relative contents of magnetite in the soils and in the underlying coral indicate that the soils are not wholly derived from coral and are probably largely derived from pumice and meteoric dust trapped in a former tidal lagoon. Spectrographic evidence supports this.

Radioactivity up to thirty times greater than that of average New Zealand soils is due largely to ionium and radium 226 out of equilibrium with parent uranium. The ionium and radium have been trapped from sea water by reef sediments, in the former lagoon. Radioactivity measurements and related data suggest that emergence of the island from the sea occurred less than 200,000 years ago.

Further study could aid in establishing the geological ages of terrace levels, thus providing a time scale for Pacific zone fossils, and in assessing effects of radioactivity on plants and animals.

Introduction

Niue Island is a very isolated island 13 miles long and 11 miles wide lying in the Pacific Ocean at latitude 19° 00′ S. and longitude 169° 50′ W. According to Yuncker (1943, quoted by Schofield 1959) it is surrounded by great ocean depths with no suggestion of past geological relationship or land connection with other island groups. Niue is about 300 miles from the nearest island and more than 2,000 miles from Australia, the nearest land mass. It is situated about 1,400 miles north-east of New Zealand.

According to Schofield (1959) Niue Island consists solely of limestone in the form of emerged reefs. The uppermost reef terrace at a height of 180 to 215 ft encloses a central basin interpreted as a former lagoon.

Soils and agriculture of Niue have been described in a bulletin prepared by A. C. S. Wright and F. Van Westendorp (in press). There is a thin mantle of soil on the island with an average depth of about 14 inches, and there are many outcrops of limestone. Soils of the elevated coastal fringe

have low silicon content and some characteristics resembling those of soils derived from volcanic ash (Grange 1949). Considerable interest therefore centres on the question of the origin of the soils, that is the extent to which they are derived from limestone and the extent to which they have received accessions of dust, or other material. According to Mr A. C. S. Wright (pers. comm.) the three main soil types on the island differ considerably in their agricultural behaviour but their morphological differences (apart from colour) are small, and mineralogical examination was therefore requested.

It was observed by one of us (M.F.) in 1955 that Niue Island soils were many times more radioactive than the average of New Zealand soils. Some account of this was given in a paper by Marsden, Ferguson, and Fieldes (1958) and there is at present considerable interest in the origin and significance of this radioactivity.

For the above reasons Niue soils have been examined by a number of physico-chemical methods and the results obtained are discussed below.

METHODS

Both finely ground whole soils and clay fractions were examined by X-ray powder diffraction using a Philips Geiger X-ray spectrometer with FeK_α radiation. In addition, some clay fractions were examined by differential thermal analysis using furnaces similar to those described by Berkelhamer (1944), with heating rate approximately 10°C per minute, stainless steel sample blocks, chromel alumel differential thermocouples, and temperature recording of the metal block temperature.

Radioactivity measurements were made initially by measuring the γ radiations on a scintillometer using about 500 g of each soil in a glass bottle placed against the end of the probe. Measurements were also made of α and β emissions of a number of samples, using suitable counting apparatus.

Magnetic susceptibilities were determined by Dr T. Hatherton of the Geophysics Division of D.S.I.R. The presence or absence of magnetite in the soils was also tested simply by stirring the air-dry soils with the blade of a magnetised screwdriver and observing the sharp edge of the blade for adhesion of magnetic particles. In addition, magnetite was separated by heavy mineral separations of some samples and its identity confirmed by X-ray diffraction. Spectrographic determinations were made by direct arcing of finely ground samples previously ignited at 500°C and mixed with an equal weight of carbon powder. Their spectrograms were compared with those of suitable standards.

RESULTS

X-ray Diffraction

Table 1 lists locality, relative radioactivity, and approximate levels of crystalline constituents of the soil types examined. The crystalline constituents (except for magnetite) were determined from X-ray diffraction patterns of the whole soils.

TABLE 1—Radioactivity and Mineral Constituents of Niue Island Soils

Soil Type No.	Sample No.	Soil Type	Locality	Relative Radio-activities (Counts per second above background)		Gibbsite	Goethite	Crandallite	Haematite	Boehmite	Calcite	Dolomite	Kaolin	Magnetite
				γ	β									
1a	5436	Hikutavake-Hakupu complex	Hakupu	15	*	*	*	*	*	*	*	*	*	*
2	5423B	Hakupu silt loam	Liku	*	*	–	m	ss	–	m	–	–	–	d
	5423D		„	5	*	tr	m	ss	–	–	–	–	–	d
2b	5437A	Hakupu very rocky silt loam with very many limestone outcrops	Hakupu	10	33	–	m	ss	–	m	–	–	–	d
	5437B		„	15	40	–	m	ss	–	m	–	–	–	d
	5435A		Kofika forest	20	8	s	s	m	?	m	–	–	?	d
	5435B		„	20	9	s	s	m	d	?	–	–	–	d
	5435C		„	20	4	s	s	?	m	–	?	–	–	d
3	5433A	Fonuakula silty clay loam	Namui Rd	40	43	s	s	m	m	?	–	–	–	d
	5433B		„	70	67	s	s	m	m	?	–	–	–	d
	5433C		„	70	62	s	s	m	w	?	–	–	–	d
	5433D		„	70	65	s	s	m	d	d	–	–	d	d
3a	5438	Fonuakula stony silty clay loam, shallow phase	Alofi-Avakele Rd	0	3	m	m	–	m	–	d	d	–	d
3b	5425A	Fonuakula rocky silty clay loam	Fuata	*	8	s	s	m	–	–	–	–	–	d
3c	5422B	Fonuakula very rocky silty clay loam with many limestone outcrops	Tumufa	70	57	ss	m	m	–	?	–	–	–	d
	5422C		„	100	100	ss	m	m	–	–	–	–	–	d

3d	5426A	Fonuakula gravelly silty clay loam with fine ironstone gravel	Fatio airstrip	10	16	ss	s	d	w	–	–	–	–	d
	5426B		,,	20	15									
	5426D		,,	30	14	ss	s	d	w	–	–	–	–	d
	5426F		,,	0	0	–	–	–	–	–	ss	s	–	–
4	5427A	Palai clay loam	Palai	30	33	ss	m	m	w	–	–	–	–	d
	5427B		,,	40	40	ss	m	m	w	–	–	–	–	d
4a	5429A	Palai rocky clay loam with many limestone outcrops	Futuna	50	26	ss	m	m	–	?	–	–	–	d
	5429B		,,	50	34	ss	m	m	–	?	–	–	–	d
	5434B		Tukuofe	10	*	s	s	m	–	–	–	–	–	d
	5434C		,,	5	16	s	s	m	d	d	–	–	–	d
	5434D		,,	0	4	s	s	s	w	m	–	–	w	d
4b	5428C	Palai very rocky clay loam	Palai	30	37	s	s	m	w	–	–	–	–	d
	5428D		,,	40	49	s	s	m	w	–	–	–	–	d
4c	5430A	Palai gravelly clay loam with fine ironstone nodules	Futuma	30	36	s	s	?	?	–	–	–	–	d
	5430B		,,	40	41	s	s	?	?	–	–	–	–	d
	5430C		,,	60	53	s	ss	?	d	–	–	–	–	d

NOTE- ss = dominant, s = much, m = considerable, d = definite, w = weak, tr = trace, ? = doubtful, – = not detected.
* = insufficient sample.

In column 2, letters A, B, C, D, etc., after sample no. indicate successive horizons at increasing depth. Numbers without letters represent shallow soils.

It is known from fusion analyses such as those given by Schofield (1959), and Wright and Van Westendorp (in press), that the total silicon content of Niue Island soils is extremely low, being commonly considerably less than 1%. The major constituents include aluminium and iron. Phosphorus, calcium, and titanium, are commonly in excess of silicon.

As expected, therefore, the X-ray diffraction patterns showed high amounts of crystalline oxides and hydrous oxides of aluminium and iron. Many of the samples were found to contain considerable amounts of gibbsite (γ–$Al(OH)_3$ and goethite (α–FeO.OH). Magnetite (Fe_3O_4) found by magnetic methods in most of the soils was identified by the X-ray method after concentration. Haematite (α–Fe_2O_3), and boehmite (γ–AlO OH) were detected from diffraction patterns of some soil samples. In addition most of the samples contained considerable amounts of crandallite ($CaAl_3(PO_4)_2(OH)_6$). Calcite ($CaCO_3$) and dolomite ($MgCO_3$) were detected in some soils containing free limestone, and there was a little kaolin in two samples.

Table 2 lists the spacings (d) and relative intensities (I) of the clay fractions less than 2 μ in equivalent spherical diameter, that had been separated from three of the soils by sedimentation. Values for crandallite, gibbsite, goethite, haematite, and magnetite are listed for comparison. Values for crandallite are those given on an A.S.T.M. card for a sample from Paraiba, Brazil, reported by J. Murdoch, University of California. Values for gibbsite, goethite, haematite, and magnetite are derived from those given for these substances in Brindley (1951).

It will be seen that in these samples spacings of strong diffraction lines relatively free from interference occur at about 2·94Å for crandallite, 4·34Å for gibbsite, 4·15Å for goethite, and 3·67Å for haematite. It is possible to make an approximate estimate of amounts of substances present from consideration of the intensities associated with appropriate spacings.

Hakupu soil clay (sample 5423D) contains much crandallite, considerable goethite, only a little gibbsite, and possibly some haematite. Hakupu soil clay (sample 5435B) also contains considerable crandallite and goethite as well as much gibbsite and probably considerable haematite. Fonuakula soil clay (sample 5426B) contains much gibbsite and goethite, relatively low crandallite, with haematite not detected. Compositions of the corresponding whole soils summarised in Table 1 are similar.

Differential Thermal Analysis

Differential thermal patterns of less than 2 μ fractions separated from Hakupu (sample 5423D and 5435B), Fonuakula (sample 5426D) and Palai (sample 5430C) soils after hydrogen peroxide treatment are shown in Fig. 1.

These patterns were obtained under comparable conditions. The large endothermic peaks near 300°C are due mainly to gibbsite. Relative sizes of these peaks suggest that for these three samples gibbsite increases from Hakupu through Fonuakula to Palai, a trend confirmed by X-ray evidence. Endothermic effects near 380°C are due to goethite.

TABLE 2—X-ray Diffraction Spacings and Intensities of Soil Clays from Niue Soils with Values for Crandallite, Gibbsite, Goethite, Haematite, and Magnetite for Comparison

Fonuakula 5426B		Hakupu 5435B		Hakupu 5423D		$Ca(Al)_3(PO_4)_2(OH)_6$ Crandallite		$Al(OH)_3$ Gibbsite		FeO(OH) Goethite		Fe_2O_3 Haematite		Fe_3O_4 Magnetite	
d	I	d	I	d	I	d	I	d	I	d	I	d	I	d	I
–	–	–	–	–	–	6.73	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	6.05	–	–	–	–	–	–	–	–	–
–	–	5.67	(13)	5.68	(13)	5.70	(40)	–	–	–	–	–	–	–	–
4.80	(100)	4.87	(100)	4.82	(56)	4.86	(30)	4.83	(100)	4.96	(30)	–	–	4.85	(30)
4.34	(42)	4.38	(58)	4.36	(7)	–	–	4.34	(60)	–	–	–	–	–	–
–	–	–	–	4.29	(7)	–	–	–	–	–	–	–	–	–	–
4.11	(59)	4.11	(43)	4.11	(43)	–	–	–	–	4.15	(100)	–	–	–	–
–	–	3.68	(20)	3.69	(5)	–	–	–	–	–	–	3.67	(70)	–	–
3.59	(6)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3.50	(4)	–	–	3.50	(25)	3.50	(40)	–	–	–	–	–	–	–	–
3.30	(27)	3.33	(20)	3.33	(10)	–	–	3.30	(30)	3.36	(30)	–	–	–	–
3.19	(9)	–	–	–	–	–	–	3.16	(20)	–	–	–	–	–	–
3.11	(6)	–	–	–	–	3.09	(5)	3.09	(10)	–	–	–	–	–	–
2.97	(9)	2.95	(20)	2.94	(100)	2.94	(100)	–	–	–	–	–	–	2.96	(60)
–	–	2.77	(10)	2.82	(7)	2.87	(5)	–	–	–	–	–	–	–	–
–	–	2.70	(30)	2.69	(17)	2.69	(10)	–	–	2.67	(60)	2.69	(100)	–	–
2.64	(18)	–	–	2.65	(17)	–	–	–	–	2.56	(30)	–	–	–	–
2.51	(6)	2.51	(16)	2.52	(8)	2.52	(5)	–	–	2.51	(10)	2.51	(80)	2.52	(100)
2.48	(12)	–	–	2.49	(3)	–	–	2.45	(50)	2.47	(30)	–	–	–	–
2.39	(80)	2.39	(57)	2.40	(40)	2.43	(10)	2.37	(50)	2.43	(70)	–	–	2.42	(10)
2.20	(21)	2.20	(30)	2.20	(13)	2.23	(20)	2.24	(20)	2.24	(40)	2.20	(70)	–	–
2.165	(24)	–	–	2.16	(50)	2.16	(50)	2.157	(30)	2.175	(40)	–	–	–	–
–	–	–	–	–	–	2.11	–	–	–	–	–	–	–	2.093	(50)
2.04	(18)	–	–	–	–	–	–	2.039	(40)	–	–	2.067	(10)	–	–
1.986	(12)	1.99	(13)	1.98	(7)	1.99	(5)	1.986	(30)	1.997	(10)	–	–	–	–
1.92	(11)	1.92	(13)	–	–	1.936	–	1.909	(30)	1.908	(20)	–	–	–	–
–	–	1.90	(13)	1.892	(37)	1.932	(40)	–	–	–	–	–	–	–	–
–	–	–	–	–	–	1.84	–	–	–	–	–	1.833	(70)	–	–
1.798	(12)	1.80	(10)	–	–	1.804	–	1.797	(40)	1.788	(20)	–	–	–	–
1.75	(12)	1.75	(12)	1.748	(33)	1.75	(40)	1.743	(40)	1.762	(10)	–	–	–	–
1.682	(33)	1.70	(30)	1.688	(25)	–	–	1.677	(40)	1.709	(60	1.688	(80)	1.711	(40)

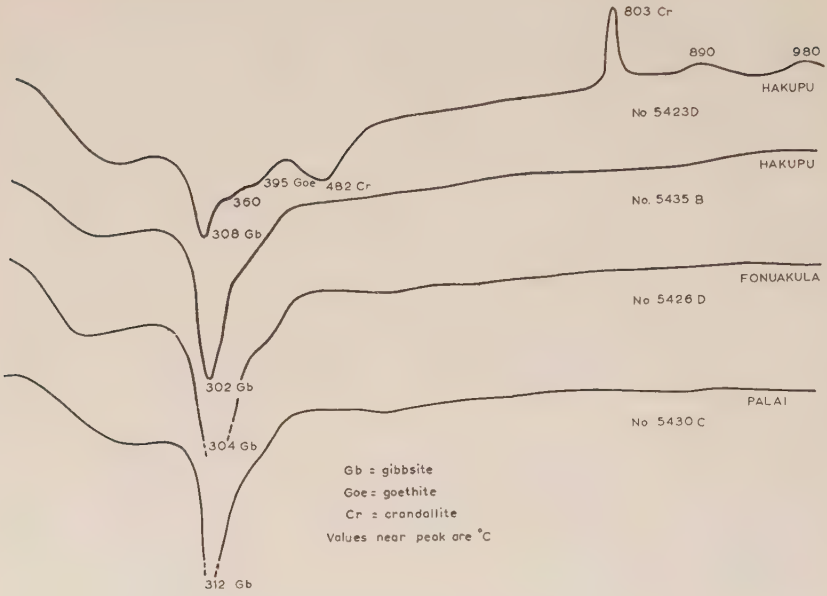

FIG. 1—D.T.A. patterns of Niue soil clays.

Sample 5423D is known from X-ray evidence to contain much crandallite and the endothermic effects at 482°C and exothermic effect at 803°C are due to crandallite. The positions of these peaks differ somewhat from those shown in a pattern published by Norrish (1957) for crandallite, and no exothermic peak appears near 920°C. Further work is needed to determine whether the discrepancy is due to differences of method or of sample.

Radioactivity

In 1955 a survey was made with a scintillometer of soil samples, held at Soil Bureau, for possible high γ activity which might point to sources of uranium. Soils from more than six thousand profiles from many parts of New Zealand and the Pacific were examined. For most soils the radioactivity was in the normal range for New Zealand soils and relatively low, but almost all Niue Island soils had γ ray activities very much above normal. All of the Niue samples tested were collected in 1949 before extensive atom bomb tests took place and artificial radioactivity is thus not a factor.

Beta activities were then checked. The β activities of the most highly radioactive soils were found to be comparable with pure potassium chloride, although the potassium content of all soils was very low (less than 0·1%).

Values of γ and β activities of a wide range of Niue Island soils are given in Table 1. Comparable γ values for normal New Zealand soils would be 0, and normal β values on a comparable scale would be between 1 and 3, mostly due to potassium. Low values (Table 1) are given only by soils very close to the underlying coral limestone and either mixed with

limestone or derived from it. It will be seen that β activities in general show trends similar to γ activities, though there are some discrepancies. Gamma activities and β activities not due to potassium are of the order of 100 times those of normal soils.

In soils of highest radioactivity the β activity, judged from data supplied by Gibbs and McCallum (1955), could be accounted for by about 0·04% of U_3O_8 in equilibrium with its daughter products, but uranium content determined analytically was much less than this value. The main activity is thus not due to uranium, and the amount of uranium in Niue Island soils has no commercial significance.

Total thorium was also low. It was not detectable spectrographically and much less than the 0·2% of thorium 232 which would be required to account for the β activity.

The rate of recovery of β emanation after disturbance of the soils (by heating or by solution in hydrochloric acid and precipitation) was checked. The recovery time of several days pointed to the main β activity being associated with build-up of radon 222 (half life 3.82 days) derived from radium 226. Radon gas is lost during disturbance of the soil and it accumulates again to a maximum in a time determined by its rate of disintegration. An accurate rate-of-recovery curve obtained by Mr G. J. Fergusson on a low background β-counter in the Nuclear Science Division confirmed that the recovered activity was due to build-up of radon 222 formed from radium 226 present in the soil, and it was presumed that ionium (thorium 230), the immediate parent of radium 226, was also present.

Alpha particle activities of the two most radioactive soils were checked by Sir Ernest Marsden who reported that the activities were about 30 times those of average New Zealand soils. Results of pulse analysis on these two soils by Mrs K. M. Glover of the U.K. Atomic Energy Authority, Harwell (pers. comm.) confirmed the presence of thorium 230 and radium 226 as the main source of activity and also confirmed that these elements were present in amounts considerably above the normal equilibrium ratios to uranium 238. They showed in addition that the ratio of radium 226 to thorium 230 was less than the equilibrium ratio.

It will be seen from Table 1 that nearly all of the Niue soils have considerable radioactivity. Upper horizons tend to be lower in radioactivity than subsoils, suggesting that topsoil conditions mobilise radium, which might be expected, and which is consistent with the low equilibrium ratio of radium 226 to thorium 230. Low radioactivity of New Zealand topsoils relative to corresponding subsoils has been noted by Gibbs and McCallum (1955).

Niue soil samples of low radioactivity included a sample of weathered coral limestone (sample 5426F), a shallow soil containing calcite and dolomite (sample 5438) and a subsoil that contained little free limestone (sample 5434D). These samples were taken from close to the underlying coral limestone. It was therefore of interest to determine the radioactive content of the limestone in relation to the soils.

100g samples of limestone from each of six quarries on the island were heated to drive off carbon dioxide and then dissolved in hydrochloric acid. There was very little residue. Sufficient ferric chloride was added to produce a residue of about 1g when the solution was treated with ammonia to bring down iron, thorium, and radium. Residues obtained in this way showed slight β radioactivity which increased with time after treatment at a rate consistent with radioactivity being due to accumulation of radon 222 from radium 226.

At equilibrium the R_2O_3 residues from quarry limestone from Avatele, from Hikutaveke Hill, and from between Matalave school and Namukulu, after allowance for the diluent effect of added iron, gave β counts comparable with those of the more active soils. Radioactivities of R_2O_3 residues of other limestone samples, however, although above background, were quite low. Replicates on 100g samples of limestone were far from reproducible, due to uneven distribution of radioactivity through the limestone, and some small chips of limestone observed directly gave appreciable counts while count rates of others of comparable size from the same sample were not above background. It was thought that activity might be concentrated in dark-coloured fossil-like inclusions in the limestone but these when separated were found to have very low radioactivity.

While it seems possible from these results that the coral limestone could have contributed radioactivity to the soils, the likelihood is that the radioactivity detected in the limestone samples was carried into it by movement of active constituents out of a radioactive soil mantle. Radioactivity values (column 5, Table 2) indicate that downward movement of radioactive elements may have occurred in Niue soils. Uneven distribution of radioactivity in the coral could be due to similar movement of radioactive elements into porous zones of the limestone. The quarry limestones analysed were taken from material that has lain beneath soil and quite close to it. The presence or absence of radioactivity in samples of deeper limestone, if these could be obtained, would help to clarify this question.

Beta count rates were obtained of clay fractions less than 2 μ separated from four of the soils. Radioactivity of each clay fraction after equilibriation was considerably higher than that of the corresponding soil. Ratios are indicated in Table 3. Concentration of radioactive materials on soil clays by virtue of their large adsorptive surfaces has been discussed by Hoogteijlung and Sizoo (1948). Adsorption seems an important factor in the high activity of the Niue soils.

TABLE 3—Ratio of Radioactivity of Clay Fraction to that of Whole Soil for Four Niue Island Soils

Soil Type No.	Sample No.	Relative Activity of Whole Soil	Radioactivity of Clay Fraction : Radioactivity of Soil
2	5423D	5	2.0
2b	5435B	20	4.7
3d	5426D	30	1.3
1c	5430C	60	1.3

Magnetic Properties

With the exception of the sample consisting mainly of limestone, all of the soils tested were shown by the simple magnetic test described above to contain magnetic particles demonstrated by X-ray diffraction to be mainly magnetite. With the exception of maghemite mentioned below other magnetic minerals were not detected. Magnetite may be formed in various ways but its formation in soils as a secondary mineral does not appear to have been reported and it is assumed that the magnetite in these soils is of primary origin.

Susceptibilities of a number of Niue whole soils were measured and compared with the value of a comparable volume of silica sand containing 2·5% of finely ground magnetite, from which approximate contents of magnetite were computed. Results are given in Table 4.

TABLE 4—Magnetic Susceptibility and Probable Magnetite Content of Different Niue Island Soil Types

Soil Type No.	Sample No.	Magnetic Susceptibility $\times 10^{-6}$	Probable Approximate Magnetite Per Cent
2	5423B	430	0.8
2b	5437B	422	0.8
3	5433B	1,100	2.1
3d	5426D	505	0.9
4a	5429B	717	1.6
4c	5430B	1,140	2.1
–	Quartz + 2½% magnetite	1,330	–

Samples of coral limestone freed from surface contamination were ground by pestle and mortar to pass a 76 μ sieve, and subjected to magnetic separation. Small amounts of dark magnetic material presumed to be magnetite were separated. The method recovered added magnetite. Yields of magnetite from several samples of limestone were of the order of one part per million. If the present day soil mantle, about 1 ft thick, had derived all its magnetite (about 1%) from coral of this type, solution of about 10,000 ft of limestone would have been required. Schofield (1959), however, suggests 55 ft as the maximum depth of limestone likely to have been removed by solution since emergence. Thus magnetite derived from coral has probably not contributed significantly to the magnetite in the soils.

Spectrographic

Table 5 lists spectrographic results for a Niue soil, Niue island sand, and Niue coral limestone. Results for coral sands from Cook Islands and Fiji are included for comparison. From Table 5 it can be seen that contents of Ca, Mg, Sr, and Ba decrease between the coral limestone and the soil, while Fe, Al, V, Cr, Mn, Ni, Co, Zr, and Si increase. It is probably signifi-

cant that in these respects the coral sands of Niue resemble soil more closely than do the coral sands of Cook Islands and Fiji. This suggests that inorganic material not derived from coral has reached the soils of Niue Island.

Spectrographic examination was also made of the clay fraction of the Hakupu soil sample (5423D) that was high in crandallite. Norrish (1957) showed the presence in some Norfolk Island soils of phosphates isomorphous with crandallite, with similar X-ray diffraction patterns, in which calcium was replaced by elements such as barium, strontium, and rare earths. There was no evidence from the spectrograms of the Niue clay of major amounts of elements likely to replace calcium. Calcium, of course, is present and as the appropriate X-ray diffraction lines of Niue Island soils correspond closely with those of crystalline crandallite, extensive isomorphous replacement of calcium seems unlikely, although it is possible that minor amounts of other elements may be included in its structure.

DISCUSSION

Mineral Constituents and Soil Type

A dominant feature of these soils which has been brought out in other work (Grange, 1949) is their low content of silicon. Because of this, unlike most soils, the Niue soils do not contain reserves of plant nutrients in unweathered primary silicates. Similarly, because of the low silicon, appreciable reserves of potassium cannot be held in layer-type silicates such as micas or hydrated micaceous materials, nor can the latter be active in fixing added potash.

Advanced weathering of Niue Island soils is shown by their high contents of oxides of iron and aluminium. It is common for such soils under high rainfall to have high phosphate-fixing properties and to require phosphate even though their total phosphate is high. The reasonable availability of phosphate of most Niue soils (Wright and Van Westendorp, in press) is associated with the presence of high calcium and with pH values near neutrality. These conditions have led to formation of considerable amounts of crandallite.

Crandallite was highest in samples of Hakupu silt loam (samples 5423 B and D). Total phosphate in the air-dry clay fraction of sample 5423D, determined by Dr W. H. M. Sanders, was 14·4% P_2O5 which corresponds to about 42% crandallite. From X-ray evidence the whole soils of this profile are comparable in crandallite content.

Some Hakupu soils contain considerably more crandallite than Fonuakula and Palai soils. Fusion analyses of Fonuakula soils and clays give values of P_2O_5 between 2·02% and 2·88% corresponding to up to about 8·3% of crandallite. From X-ray evidence the range of crandallite content in Fonuakula and Palai samples appears comparable. Both types contain much less crandallite in classes containing ironstone nodules or gravel, and in Palai gravelly clay loam (samples 5340 A to C) crandallite could not be detected definitely in X-ray patterns of the whole soils.

TABLE 5—Spectrographic Analysis of a Niue Island Soil Profile, Coral, and Coral Beach Sands

Sample	Si	Mg	Na	K	Ca	Fe	Al	V	Cr	Mn	Ni	Co	Sr	Ba	Zr	Ti	Cu
Niue soil No. 5426A	<3	0.5	0.1	0.5	1.5	15	25	200	1500	3000	10	25	250	<30	200	–	–
,, 5426B	<3	0.5	0.1	<0.5	1	15	25	200	1500	3000	10	25	220	<30	200	–	–
,, 5426C	<3	0.5	0.1	<0.5	0.8	15	25	200	1500	3000	10	25	200	<30	200	–	–
,, 5426D	<3	0.5	0.1	<0.5	0.8	15	25	200	1500	4000	10	25	150	<30	200	–	–
,, 5426E (Coral sand)	0.4	1	0.1	<0.5	>10	1	2	10	30	30	<1	<1	1500	150	<30	500	5
Coral from Niue	0.3	5	0.1	<0.5	>10	0.01	0.2	<10	<3	<30	<1	<1	10000	150	–	–	2
Coral sand from Cook Islands	0.3	2	0.7	<0.5	>10	0.2	0.4	<10	3	<30	<1	<1	20000	200	<30	300	3
Coral sand from Fiji	1.5	1.5	0.5	<0.5	>10	0.4	1	30	1	30	<1	<1	20000	200	<30	800	15
	%							p.p.m.									

The evidence as far as it has been taken suggests that phosphate content of Hakupu soil types is higher than that of the other soils. Crandallite is the stable phase of phosphate present and, in the presence of sufficient calcium, crandallite has formed up to the limit of the phosphorus present. Thus Hakupu soils (Samples No. 5423 B and D) that are high in phosphorus contain over 40% of crandallite and little gibbsite. Almost all of the aluminium in these soils is in the form of crandallite.

In a valuable paper on forms of soil phosphate Norrish (1957) reported the presence of crandallite in the coarser fractions of certain soils including a Cook Islands soil (sample 5810), the clay fraction of which had previously been examined at this laboratory. Re-examination of various fractions of the latter soil confirmed the previous finding that the samples were low in crystallinity, possible evidence for crandallite being confined to a broad ill-defined line near 2·94Å which could have been caused by magnetite (see Table 2), maghemite, or other possible constituents. In order to concentrate crandallite, Norrish heated the materials with hydrofluoric acid but this severe acid treatment and the reactivity of aluminium and calcium with fluoride suggest that conclusions regarding the residue should be treated with caution. This soil and two others in which Norrish identified crandallite were derived from parent materials of basaltic origin, and one of the parent materials also contained coral limestone.

Norrish (1957) tentatively suggested that large particles of crystalline crandallite might be associated with the low amounts of "available" phosphate reported in Cook Islands soils of high total phosphate (Grange and Fox, 1953). The Cook Islands soils referred to contained total phosphate 1 to 4% P_2O_5 and "available" phosphate of the order of 0·004% P_2O_5 determined by chemical means. In the Niue Island soils "available" phosphate determined by the same method is as high as 0·076% P_2O_5 in sample 5423B which contained approximately 40% of well crystallised crandallite. Furthermore, the results of agricultural observations by Wright and Van Westendorp (in press), show that most of the soils are well supplied with phosphate as far as plant growth is concerned. The least satisfactory in this respect appears to be soil type 4C in which phosphate availability is described as fair to moderate. In a type profile of the latter (samples 5430 A to C) crandallite could not be detected with certainty while the "available" P_2O_5 ranged from 0·160 to 0·017% down the profile. There is no satisfactory correlation between the mineralogy of Niue soils and their phosphate availability but it seems that the presence of large amounts of well crystallised crandallite does not imply low availability of phosphate either chemically or to plants.

In general, subsoils of Hakupu, Fonuakula, and Palai soil types tend in colour to be brown, red-brown, and red respectively. It might be thought that haematite or some other coloured iron compound would be present in increasing amounts with increasing redness. As seen from Table 1, however, all of the soils contain considerable goethite and usually some magnetite while amounts of haematite detected by X-ray diffraction are variable. Thus, the colour differences appear to be due to surface and particle size effects rather than to nature of the bulk of the crystalline iron oxides.

Evidence Concerning the Origin of the Soil

Grange (1949) observed the lateritic properties of these soils lying over limestone and pointed out the presence of magnetite, and the relatively greater titania in the soils with respect to insoluble matter from the limestone. For these reasons he considered that volcanic material had influenced the nature of the soils.

It was suggested by Mohr and van Baren (1954) that further mineralogical analyses might give definite proof of accession of volcanic material. However, the above results show that, as indicated by the low silicon content, weathering is so advanced that the resistant mineral magnetite is the only mineral detected that could be of primary origin. Minor amounts of maghemite, γ Fe_2O_3 were found in light mineral separates of the magnetic fractions of some of the soils. Maghemite, a comparatively rare mineral, is considered by Rooksby (in Brindley 1951) to be probably an alteration product of magnetite.

Grange (1949), discussing soils of the Solomon Islands, pointed out that soils on terraces underlain by limestone varied according to amounts of beach deposits left on them and of volcanic ash received later. Generally the lowest terraces had profiles resembling rendzinas and the higher terraces containing more volcanic ash were friable and lateritic. Zans (1953) (reported by Mahr and van Baren, 1954), discussed the origin of bauxite on limestone in Jamaica, agreeing with Goldich and Bergquist that the ultimate source of the lateritic constituents was volcanic ash or pyroclastic material of intermediate (andesitic) composition.

From evidence of this type it appears that lateritic soils of low silica content would form under tropical island conditions where volcanic ash was deposited on coral limestone. The presence of magnetite in Niue Island soils supports the possibility of contributions by volcanic material. Magnetite content of underlying limestone is very low and as pointed out by Grange (1949), magnetite is common in volcanic material as a primary mineral. It is not common as a secondary mineral in soils. It is clear from the presence of nodules in some Niue Island soils and not others that different soils have been formed by different natural processes. The presence of magnetite in all Niue soils (Table 1) in spite of differences in soil processes makes it still more unlikely that the magnetite is of secondary origin and further supports the suggestion of accessions of volcanic material.

From the shape of the island it is likely that floating pumice trapped in the old lagoon contributed to its reef sediments. Accessions of volcanic material from this source together with meteoric dust also largely of volcanic origin may account for a considerable proportion of the parent material of Niue soils.

Significance of Phosphate

It might be thought that unusual features of high phosphate in the form of crandallite, and high radioactivity would throw additional light on the origin of the soils.

Thorium and other radioactive substances are sometimes found in nature together with phosphatic minerals such as monazite (Goldschmidt, 1954) and Pacific Islands rock phosphates (Marsden *et al.*, 1958). There appears, however, to be an inverse correlation between radioactivity and crystalline phosphate in Niue soils. Soils with highest contents of crandallite and phosphate, for instance, such as the Hakupu soils (samples 5423 and 5437), have relatively low radioactivity. It appears also that radium is retained by soils with high contents of aluminium and iron oxides and high pH due to calcium may also have aided retention of radioactivity in the Niue soils.

Large variations of phosphate in the soils may point to accessions of phosphate of marine origin and the fact that the Hakupu soils, which tend to occur on an elevated fringe near the coast, are high in phosphate and crandallite could be connected with accessions from droppings of sea-birds. These possibilities, however, could not be maintained without further supporting evidence.

Significance of Radioactivity

Any interpretation of the origin of the soil needs to take account of both the nature of the radioactivity and also its intensity.

It seems clear from the rates of recovery of β activity discussed above that much of the radioactivity of Niue soils is due to radium 226. This isotope is an immediate daughter product of ionium (thorium 230) and both are daughter products of the natural radioactive element uranium 238. The normal ratio of radium 226 to uranium 238 in equilibrium with its daughter products is approximately 1 to 3,200,000. As mentioned above the activity of the most active soils could be accounted for by the presence of 4×10^{-4}g of uranium 238 (in equilibrium with its daughter products) per gram of soil. The content of radium 226 on this basis would be, in round figures, 10^{-10}g per gram of soil. The activity of the soils is consistent with the presence of up to 10^{-10}g per gram of radium 226 but the uranium content is much less than 4×10^{-4}g per gram of soil. Thus radium 226 appears to have been separated from its parent uranium 238.

Separation of uranium 238 from its daughter products occurs during marine sedimentation (Goldschmidt 1954). Products of rock weathering reaching the sea contain uranium and its disintegration products but conditions are such that uranium tends to stay in solution while its daughter products ionium (thorium 230) and radium 226 are deposited by precipitation and adsorption. Thus in recent marine sediments radium 226 is not in equilibrium with uranium. The existence of disequilibrium of this type in Niue Island soils suggests that their radioactive constituents have been derived from sea water.

The intensity of the radioactivity also requires some explanation, for as pointed out by Marsden *et al.* (1958), α activity of Niue soils is up to thirty times greater than that of average New Zealand soils.

Abyssal red clays from deep-sea sediments have been reported to be high in radioactivity due to radium out of equilibrium with other elements of the uranium family. Radium contents of up to $0{\cdot}97 \times 10^{-10}$g per gram reported

by Kroll (1955) and up to $0{\cdot}29 \times 10^{-10}$g per gram reported by Urry (1949) for deep Pacific sediments are in the same range as those found in Niue Island soils. Thus radioactivity of Niue soils is similar in nature and intensity to that of deep ocean bottom sediments and such sediments occur near the island.

The similarity is unlikely to be coincidental though its significance may be difficult to deduce. It seems that ionium may be deposited, and radium trapped on ocean sediments. Thus in waters remote from land-masses where sediments accumulate slowly their radioactivity may become relatively high if accumulation of radioactive matter proceeds more rapidly than dilution by arrival of fresh sedimentary material. The sediments need not necessarily be on the ocean bottom but might for instance, in the case of Niue Island, have consisted of reef sediments and volcanic ash deposited in shallow waters of the ancient lagoon on the island at some time during its past history.

Assuming a mechanism of accumulation of radioactivity similar to that outlined above it would be expected that, for any set of circumstances which remained sufficiently constant for a long time a maximum equilibrium value for radium might be reached which would depend on (a) the capacity of the sediment to trap ionium and radium, (b) the rate at which ionium and radium were conveyed to the sediment by circulating seawater, and (c) the rate of disintegration of these radioactive elements. The maximum value of radium found in abyssal sediments approaches 10^{-9}g per gram and although sediment in a lagoon might be exposed to a greater volume of sea water per unit time through tidal movements it may nevertheless be reasonable to assume that the maximum content of radium attained by submarine sediments is of a similar order.

It is evident from the results above that radium in Niue soils is mainly unsupported by uranium. By analogy with abyssal red clays it would be expected that the radium was at least partly supported by ionium and some results of pulse analyses mentioned above confirm this.

Assuming that the maximum amount of radium supported by ionium in Niue soils has not exceeded 10^{-9}g per gram, which may be reasonable, and taking the half life of ionium as $6{\cdot}9 \times 10^{4}$ years, the elapsed time since removal of the sediments from the sea can be estimated. For soils of radium content 10^{-10}g per gram this would be of the order of 3 half-lives or approximately 200,000 years, which would be a maximum figure.

A value for an upper possible limit of the time of removal of the sediments from the sea can also be estimated from the total lead content of Niue soils which has been estimated spectrographically as approximately 10^{-5}g per gram. If all of the lead were lead 206 derived from ionium in the present sequence of decay it would have been formed from approximately 10^{-5}g per gram of ionium. As the present level judged from radium values is of the order of 4×10^{-9}g per gram ionium, the maximum degree of reduction of ionium by decay is to about four ten thousandths of the original ionium. This degree of decay requires approximately ten half-lives of $6{\cdot}9 \times 10^{4}$ years, or approximately 700,000 years. It is unlikely that all of the lead present is as the 206 isotope or that it was all derived from the present decay cycle, so that the earliest emergence from the sea is likely to

have taken place at some time more recently than 700,000 years. This maximum time would be considerably reduced if the proportion of lead 206 to total lead were low, as might be expected. On the whole it seems unlikely that the ionium content could ever have been as high as 10^{-5}g per gram and a time of 200,000 years or less as obtained from the previous assumptions seems more likely.

Schofield (1959) by correlation of terrace levels with well established Pleistocene sea levels suggests that emergence of the higher terraces of about 115 ft to 200 ft in altitude may have occurred between 270,000 and 900,000 years ago. It would appear that further detailed work on the time scale indicated by the radioactivity of Niue soils could have value in establishing times of emergence corresponding to the different terrace levels.

Medical Significance of Radioactivity

Preliminary consideration of the medical significance of relatively high natural radioactivity of the soils is given by Marsden *et al.* (1958). Simpson (1954) discussed the possible connection of thorium (reported by one of us (M.F.) at an early stage of the present work) with incidence of cancer and abnormal lung lesions of some Islanders. He considered that while there was a possibility that inhalation of road-dust may occasionally give rise to asymptomatic pulmonary fibrosis and while some road dusts may contain considerable thorium it could not be demonstrated from evidence available that the thorium was in any way injurious to the health of the Niuean people. As discussed above, the form of thorium concerned is ionium (thorium 230) and decay of radium 226 is the source of much of the radioactivity. Radium is known to have caused lethal lung cancers in mine workers in Austria but according to Simpson (1954) there has been no recorded case of intrathoracic neoplasm of any type found on the island in the past ten years during which malignant neoplastic disease has been well recorded.

Medical effects of this level of radiation on health and genetics warrant further study. Even if, as seems possible with reference to health, the effects are not great the facts are likely to provide significant data for consideration of possible effects of radioactive fallout.

CONCLUSION

The shallowness and the advanced state of weathering of the soil mantle of Niue are such as it is difficult to determine the origin of the soils. Geological evidence suggesting contributions from volcanic material is supported by the fact that magnetite, which is present in all soil types and all soils in spite of differences in soil processes, seems unlikely to be of secondary origin, and is not derived from coral.

Spectrographic evidence of contamination of the Niue coral sands indicates that materials not derived from the coral may have reached the island. Radioactivity appears to have been derived from sea water from which the materials forming the present soils emerged at some time more recent than 200,000 years ago. The relatively lower radioactivity of Hakupu soils lying on the higher level of the coastal fringe points to their emergence from the

sea at an earlier date than the material forming the Fonuakula and Palai soils in the depressed central basin of the island which represents the site of a former lagoon (Forster (1777) quoted by Schofield (1959)).

The physico-chemical evidence is consistent with the soils having been derived from reef sediments mainly of volcanic origin. Because of the distance of the island from land masses and the great depth of the surrounding ocean the sediments have properties similar to those of abyssal red clays and, like them, must have accumulated slowly and must have received a considerable portion of their material by accessions of dust and volcanic ash.

Principal mineralogical differences through the main soils types Hakupu, Fonuakula, and Palai, are decreasing crandallite and increasing gibbsite.

Further work is needed to establish the exact nature and origin of the radioactivity of the soils and possible effects of the radioactivity on plant and animal life. It could probably also assist in establishing geological time scales for terrace levels and for fossils in the Pacific area.

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LEAF SHAPE IN *RANUNCULUS INSIGNIS*

By F. J. F. Fisher, Botany Division, Department of Scientific and Industrial Research, Christchurch.

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Summary

Variations of leaf shape in *Ranunculus insignis* Hook. f., have been found to obey the polar equation $R = K\theta$, where R and θ are polar coordinates and K a constant for each leaf. Values of K can be predicted from a graph relating relative width to the polar equation. These predicted values are sufficiently reliable expressions of actual values for the relative diameter D to be used with confidence to represent the marginal curve in studies of geographic variation of leaf shape in this species.

Introduction

Variations of biological form among related organisms often appear to require very complex systems of measurement if a detailed quantitative analysis is called for in the course of a taxonomic or comparative study. Although it was shown by D'Arcy Thomson (1917) that in almost every case relatively simple mathematical transformations are likely to be involved, so that whole systems of apparently unrelated shapes may be tied together by minor alterations in the constants of often very simple equations, in few studies have such unifying relationships been revealed. The number and kind of measurements in some investigations have been multiplied to an advanced degree without in the end uncovering a satisfactory integrating formula (Olsen & Miller 1958). For plants, the series of studies by Sinnott (1935, 1936, 1958) on fruit shape in *Cucurbita,* the work of Silow (1939) on the genetics of leaf-shape in cotton, and that of Melville (1937, 1949) on leaf-shape in the elm, have gone some of the way towards providing simplifying measures for the diversity of respective forms under study. While the importance of shape rather than absolute size has been stressed in some of these studies (see Sinnott 1958), in few of them has the shape been defined by more than simple proportions or angles.

Ranunculus insignis Leaves

In the case of the leaves of *Ranunculus insignis* Hook. f., a subalpine species of the higher mountains of New Zealand, the shape is basically heart-shaped or cardioid. These leaves are undivided, although the margins are provided with a greater or smaller number of shallow teeth or lobes (Fig. 1). The shape of the marginal curve of leaves formed by any one individual is particularly consistent even though the absolute size of any leaf is determined by the conditions applying during development. In the course of a year the coefficient of variation in size may be several times that of shape as measured by the ratio of width to length (Table 1). Successive leaves formed during the same season are very consistent, even size varying only by small amounts (Fig. 1B).

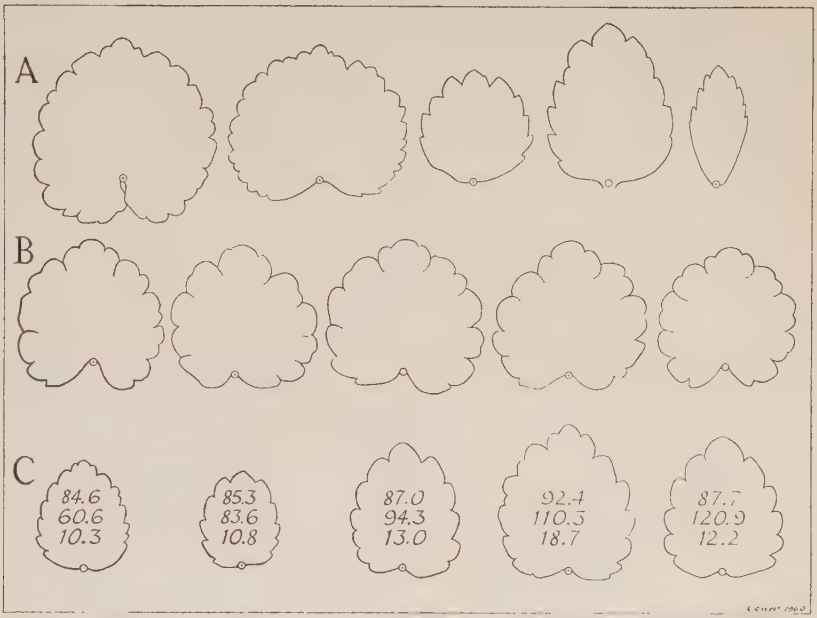

FIG. 1—A. A group of typical cardioid leaf-shapes in *Ranunculus insignis* Hook. f. from five representative localities: from left, Mts Whakere, Ruapehu, and Barron, Severn Stream and Castle Hill.

B. Leaves from one plant formed during the course of one summer in the experimental garden. Plant originally collected at the Bluff Station, Clarence Valley.

C. Five leaves having similar relative widths taken from populations having a wide range of average shapes. Upper figure: percentage ratio for individual leaf; middle figure: mean ratio for population sample of 50 plants; lower figure: standard deviation of ratios in population sample. From left to right original populations were: Four Peaks, Island Saddle, Severn Stream, Mt Fyffe and Molesworth.

TABLE 1—Measurements of five leaves taken from one plant in the course of a year in the experimental garden. The coefficient of variation for either length or width is several times greater than that for the width to length ratio. Compare with the leaves formed in a single season shown in Fig 1 B.

Leaves	A	B	C	D	E	Mean	s.d.	Coeffic. Variation
Length (mm)	86	81	65	71	78	76.2	8.3	10.9
Width (mm)	102	91	77	83	94	89.4	9.7	10.8
Width/Length %	119	112	118	117	120	117.2	3.1	2.6

Within each population of the species the marginal curve shape and hence the width-length ratio varies more widely than within leaves of one plant. But if a set of leaves having the same width-length ratio is selected from the range present in a population, it is found that these leaves are very

similar in the actual shape of their marginal curves. It need not always follow that similar ratios of width to length must correspond to the same kind of marginal curve. This is because the ratio depends on the length and not the position of the wide diameter upon the long axis. It is of course a matter of everyday experience that within a species the shape of leaf margins tends to be much less variable than between different species. In *R. insignis* each ratio does correspond to one particular form of marginal curve.

The mean shape of leaves changes from one mountain range to another. While in the north of New Zealand the average shape in each population tends to be broadly reniform, in the south it becomes increasingly elliptical (Fig. 1A). Nevertheless, in *R. insignis,* leaves having the same ratio, even when taken from widely separated populations for which the mean ratios are very different, possess remarkably similar curves (Fig. 1C). This suggests that there might be an underlying uniformity in the growth processes of such similar leaves even though the mean characteristics of the populations from which they come are so different. Such an underlying uniformity in development is usually taken for granted for different leaves of the same individual. It is also taken for granted, though with less certainty, for the leaves of different plants of a population, particularly where these different plants are similar in appearance. It would, therefore, be a convincing demonstration of the genetic continuity of a series of differing populations, and also of the similarity of growth processes throughout, if all variations in a character such as the marginal leaf-curve could be shown to bear a simple mathematical relation to each other. If such a relation were demonstrated for morphological variations, it might be inferred that the genetic differences underlying them might be correspondingly simple, and any taxonomic treatment arising out of such variation accordingly straightforward. Besides thus simplifying the description and taxonomic treatment of leaf diversity in a geographic framework, such a mathematical treatment might also contribute to an understanding of some of the processes concerned in leaf morphogenesis.

Analysis of the Marginal Curve

It was shown by D'Arcy Thomson (1917) that the shape of leaf margins is often of the form common in other biological material where increments of growth take place centrifugally around a central nodal zone, an angular component introducing the spiral form. He showed that the form taken by such growth curves is frequently that expressed by the simple polar equation $R = K\theta$ (equation 1), where R is the radius measured from a nodal point, θ the angular departure from an initial axis, and K a constant relating these coordinates in any particular individual. In some cases the relation is logarithmic thus $\log R = K\theta$, as in the shells of molluscs or the frustrules of the Foraminfera. In *Ranunculus insignis,* the marginal curve has been found to be essentially linear over most of its length when the lobe tips are plotted against angular departure from the central vein of the leaf. The equation thus becomes $1 - R = K\theta$, where K represents the slope of the relation plotted on rectangular coordinates, its value varying from leaf to

leaf. In order to facilitate the rapid comparison of large numbers of leaves, a conversion apparatus (Fig. 2) has been constructed in which a rotating table is linked mechanically to a sliding carriage. With this plotting instrument, the rectangular equivalent of the polar curve traced by a leaf margin may be quickly put on record (Fig. 3).

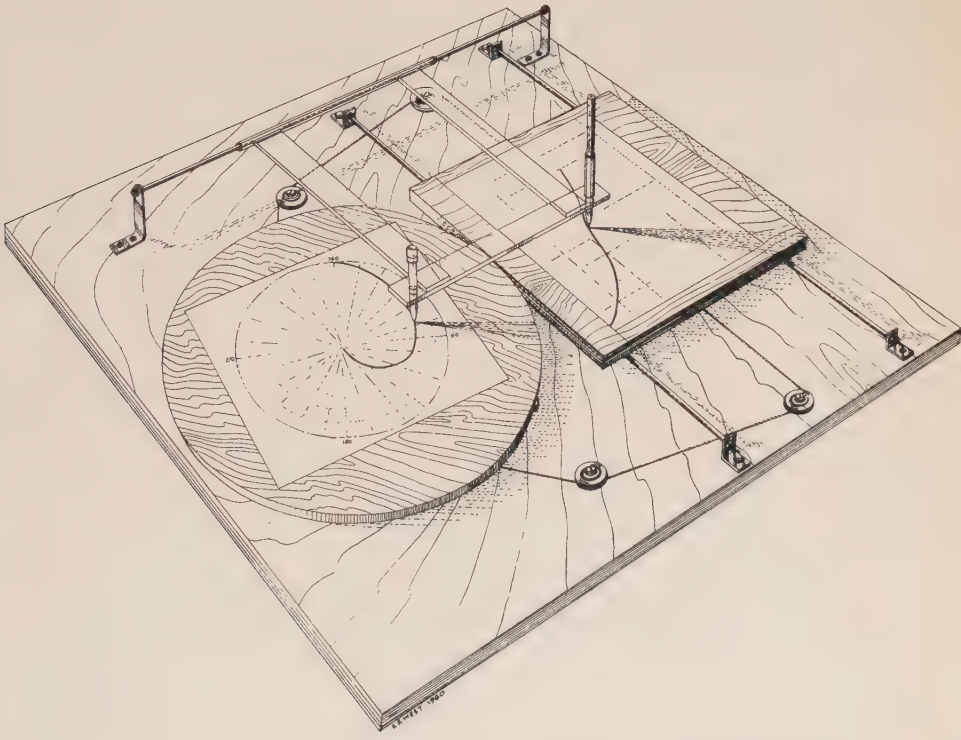

FIG. 2—Sketch of apparatus used for the interconversion of polar and rectangular coordinates. In use, the rotating table is moved with one hand while the other hand adjusts the sliding frame, so as to guide the pointer along the original curve, the new curve being drawn on the other table.

In the rectangular graphs resulting from the conversion of leaf outlines treated as polar curves, slight departures from linearity occur. An estimate was therefore obtained of the reliability of the simple linear equation to represent the observed curves. An analysis of the regression of R upon θ was obtained, using the coordinates of the lobe tips of 10 leaves from each of five samples covering a wide part of the range of variation. An estimate of linearity is given by the standard errors of the regressions in each case.

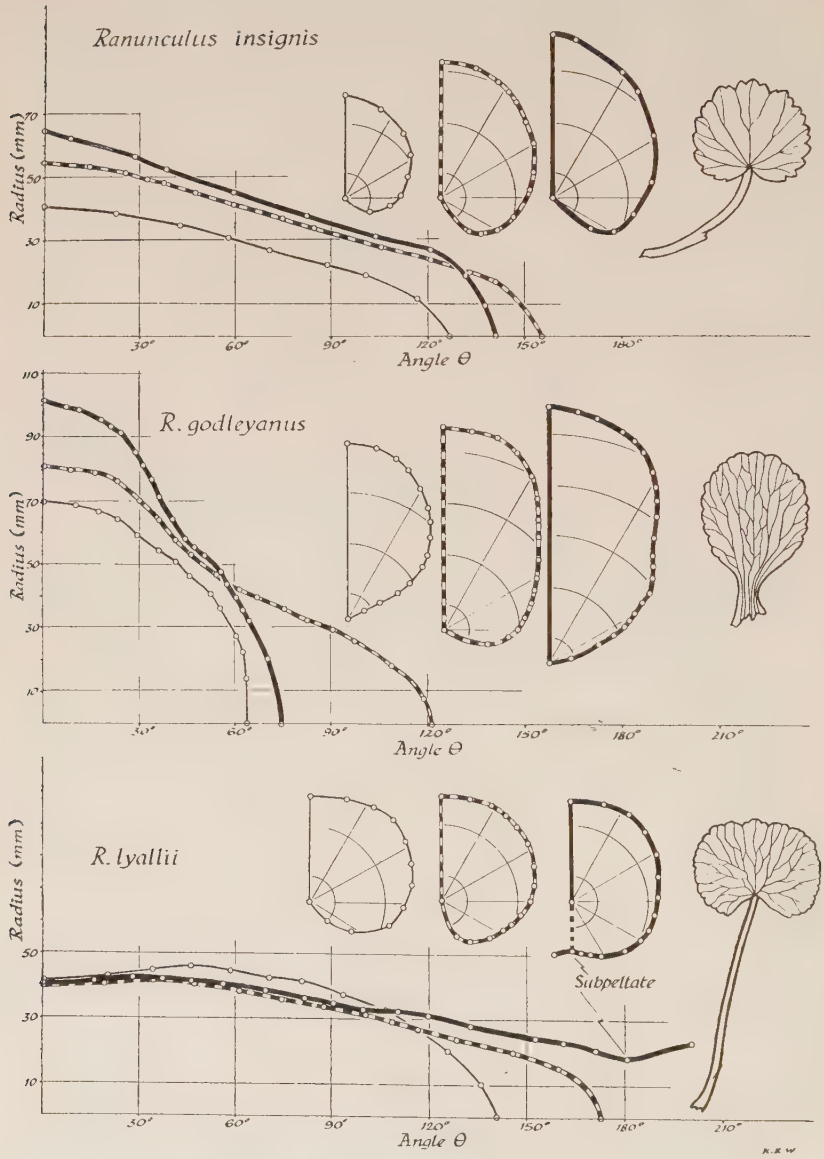

FIG. 3—Smoothed marginal curves together with their rectangular equivalents showing the essential linearity of the polar coordinates of a group of leaves of *R. insignis* compared with other species.

It will be seen that the sample means may be taken as reliably characterising the respective sets of leaves, even though the value of K in the equation $R = K\theta$ differs significantly between individuals. From this it may be concluded that a linear form of equation may be used with confidence to represent the form of the leaf margin in dealing with population samples of the species concerned (Table 2).

TABLE 2—Analysis of the regression of R upon θ fitted to a straight line. The residual error in each case unaccounted for by a linear relation is sufficiently small for the regression to be adequately represented by the straight line.

Sample 1. Mt. Princess

	d.f.	s.s.	m.s.	
Mean regression	1	17607	17607	94.56++
Het. regression	9	1676	186.2	19.20++
Error	94	915	9.7	
Total	104	20198		
Mean slope	−0.3312	±0.0341	(9 d.f.)	(weighted for error)

Sample 2. Mt. Whakere

	d.f.	s.s.	m.s.	
Mean regression	1	16806	16806	113.71++
Het. regression	9	1330	147.8	15.72++
Error	69	647	9.4	
Total	79	18783		
Mean slope	−0.3545	±0.0332	(9 d.f.)	(weighted for error)

Sample 3. Ruapehu

	d.f.	s.s.	m.s.	
Mean regression	1	8750	8750	88.56++
Het. regression	9	889	98.8	13.53++
Error	77	559	7.3	
Total	87	10198		
Mean slope	−0.2837	±0.0300	(9 d.f.)	(weighted for error)

Sample 4. Clarence Valley

	d.f.	s.s.	m.s.	
Mean regression	1	7010	7010	147.27++
Het. regression	9	428	47.6	21.64++
Error	54	119	2.2	
Total	64			
Mean slope	−0.2991	±0.0246	(9 d.f.)	(weighted for error)

Sample 5. Mt. Catherine

	d.f.	s.s.	m.s.	
Mean regression	1	8288	8288	292.8++
Het. regression	9	255	28.3	7.16++
Error	44	174	3.95	
Total	54	8717		
Mean slope	−0.5213	±0.0299	(9 d.f.)	(weighted for error)

While it has been established that the linear equation can satisfactorily represent the marginal curve in samples of leaves, it remains to be shown whether it is possible to predict the position of the curve present in any particular case from a knowledge of a pair of coordinates describing a point on the margin. If this were possible, then a simple measurement of the position of a single point, for example at the greatest width, should fix the position of the whole curve. In the case of the greatest width, which is one of the easiest measurements to make, it is unfortunate that a complex function is involved in its relation to the polar equation under examination.

While the greatest width may be expressed as a direct function of R and θ (Fig. 4) and accordingly must possess a definite value for a given value of

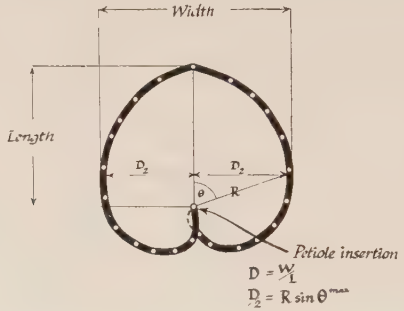

FIG. 4—The relation between greatest width of a leaf and the polar coordinates at the intersection with the marginal curve. Relative width D is the ratio of width to length expressed as a percentage.

K, the relation between the two equations involves the infinite series relating the terms sin θ and θ (equation 3).

$$D/2 = (R \sin \theta)^{max} \quad (2)$$

$$\text{but } R = K\theta \quad (1)$$

$$\text{therefore } D = (2K\theta \sin \theta) \max \quad (3)$$

In order to avoid the complications introduced by this infinite series, a set of suitable values of θ and the corresponding values of K and D have been plotted (Fig. 5). In this form it is found that the use of D as a predictor is considerably less reliable than K values calculated from the full series of marginal points. Nevertheless, for the samples studied the variation in K is sufficiently high within each sample to compensate for any weakness D may have in this regard. Mean values give a sufficiently close estimate of K for D to be useful as a statistic in subsequent studies. Since both methods, measurement of D alone, and the establishment of K directly from a regression analysis of the marginal points give similar errors, they may be used equally well for the discrimination of localities. (Table 3.)

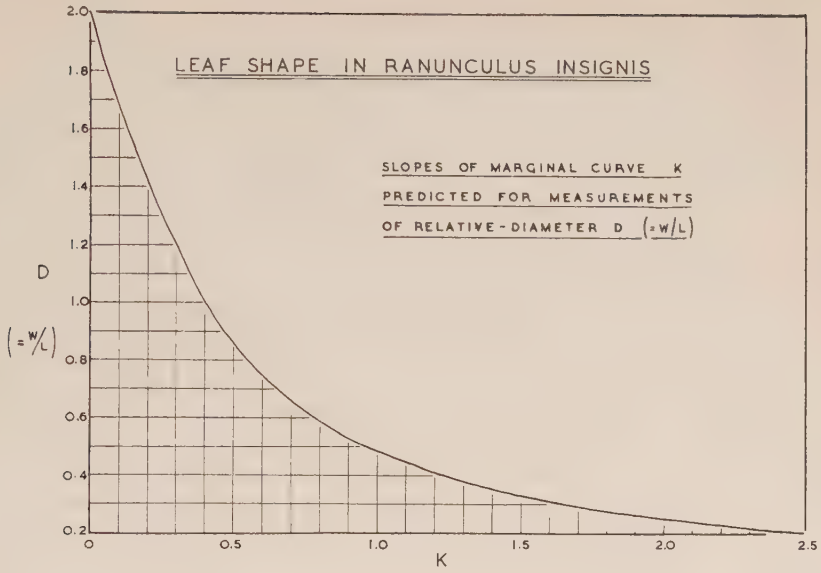

FIG. 5—Values of K in relation $R = K\theta$ for the polar coordinates of marginal lobe tips predicted from the relation shown in Fig. 4 of K to D, the maximum relative width.

TABLE 3—Values of K obtained respectively by calculation of the regression of R upon θ (left hand figures) and by prediction from the measurement of width using the graph in Fig. 5 (right hand figures).

Whakere		Princess		Ruapehu		Catherine		Clarence	
Calc.	Predict.	Calc.	Predict.	Calc.	Predict.	Calc.	Predict.	Calc.	Predict.
.3457	.26	.3641	.245	.3009	.17	.5637	.72	.4194	.565
.5237	.435	.2769	.235	.4217	.40	.4784	.56	.3994	.40
.2127	.17	.3295	.205	.5548	.325	.5906	.81	.2215	.47
.4022	.27	.3304	.29	.1938	.24	.3068	.68	.3744	.75
.3080	.28	.2890	.26	.3282	.40	.3265	.62	.3136	.485
.3656	.34	.2552	.18	.2037	.26	.5533	.715	.2980	.46
.4654	.48	.2191	.24	.2604	.285	.6215	.78	.2877	.435
.3546	.19	.3054	.205	.2510	.14	.6259	.89	.2610	.435
.5167	.245	.6365	.395	.2813	.365	.4925	.84	.3177	.555
.3825	.26	.3240	.235	.2550	.175	.3099	.80	.1454	.30
Mean .3877	.2930	.3330	.2490	.3051	.2760	.5075	.7415	.3038	.4855
s.e. .0301	.0313	.0362	.0189	.0345	.0303	.0355	.0323	.022	.0378

ACKNOWLEDGMENTS

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A DISCUSSION OF LEAF MORPHOGENESIS IN *RANUNCULUS HIRTUS*

By F. J. F. Fisher, Botany Division, Department of Scientific and Industrial Research, Christchurch.

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Summary

(1) Anomalous cyclic variations in the leaf shape of *Ranunculus hirtus* DC, which occur under wild conditions, have been experimentally reproduced by temperature treatments.

(2) Leaves produced at 10°C have 3 separate leaflets whereas those at 20° have a shallowly lobed form.

(3) Relatively thinner and larger laminae, longer petioles, and more sparing hair development are exhibited by plants grown at 20°C compared with those growing at 10°C.

(4) Almost identical differences are shown by plants grown at high humidities (98%) rather than at low (45%–50%) and also at low light intensities (200 f.c.) rather than higher (800 f.c.). Such humidity or light contrasts have, however, no effect on lobing.

(5) The hypothesis is advanced that temperature controls the pattern of distribution of limiting growth materials through a channelling of the supply through the linearly oriented cells of the procambium.

(6) No significant differentiation has been observed in the distribution of radioactive assimilates in autoradiographic sections of the young leaf primordia fixed two hours after administration of $^{14}CO_2$.

(7) The variation in leaf shape may have ecological significance by allowing the species to extend its habitat across a wider zone of the forest margin. The temperature response may trigger a change of leaf shape which adapts the species to increased evaporation rates accompanying lower temperatures, or to decreased lighting conditions accompanying higher temperatures.

Introduction

In the course of general studies of the variation and systematics of the New Zealand buttercups, certain anomalous variations of leaf shape have been found in a number of species. Notably, these species include an alpine group comprising *Ranunculus enysii, R. chordorhizos* and *R. verticillatus* together with the lowland species *R. hirtus*. Variations occur not only in the normal way, between different plants and different populations, but also between successive leaves of single plants (Fig. 1). Differences of this kind when found in other higher plants are sometimes a function of age, as in the numerous cases of juvenile to adult changes. Although such changes also occur in the *Ranunculi*, the leaves of young plants regularly being less dissected than of older ones, the variations under discussion occur in adult plants in a cyclic fashion. Again, such changes are known to be associated in some plants with the annual development of reproductive structures; in the *Ranunculi* concerned here, this relation is ruled out by variations which have been found to be just as fully developed in non-flowering as in flowering states of the plants concerned.

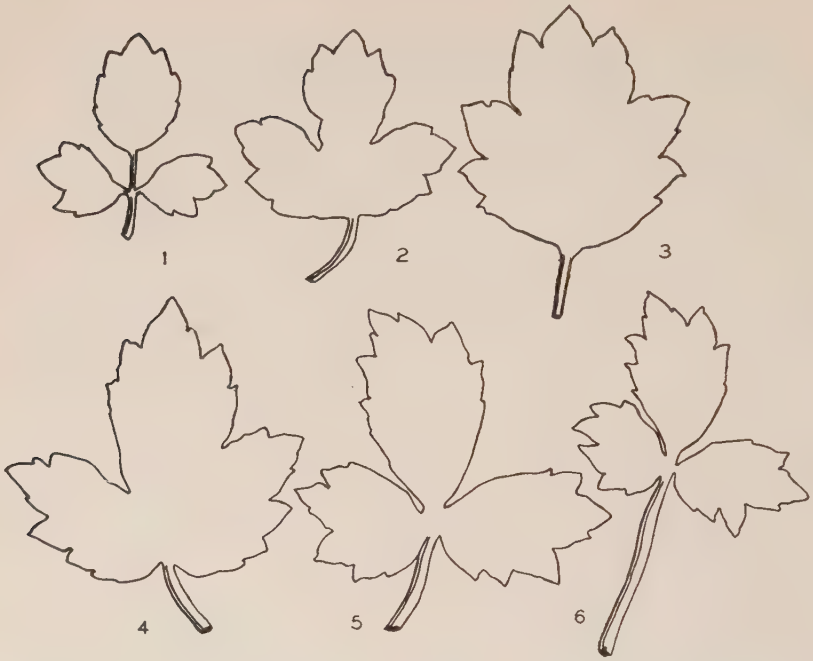

FIG. 1—A series of leaves taken from a single plant of *R. hirtus* DC in the course of a year. Leaves were harvested at intervals of approximately two months. Plants were grown outside, shielded from direct sunlight.

In the winter and early spring, *Ranunculus hirtus* habitually produces leaves more completely dissected than at other times of the year. Apparently a seasonal factor is having an effect. Among the environmental factors which are known to have a marked seasonal periodicity are light intensity and duration, temperature, and humidity. Light intensity and temperature both affect photosynthesis over a wide range. Respiration and translocation are also temperature dependent. Light duration commonly controls flowering and through dormancy mechanisms often has strong effects on vegetative development as well. Humidity is believed to be associated with leaf shape through the adaptive advantages of microphyllous leaf forms in conditions of high evaporation. Of the effects mentioned, that of temperature, in experiments by Pound (1949) on horseradish, closely parallels the effects observed in *Ranunculus,* finely dissected fern-like leaves being produced at 16°C while normal broadly laminate forms appeared on the same material at 28°C.

With these major factors in mind, experiments were initiated in an effort to discover the cause or causes of the natural variation. A pair of air-conditioned cabinets were constructed (see Fisher 1953) with temperature, humidity and lighting controllable within reasonably close limits. Humidity was chosen as the variable in the first run, the possibility of an adaptive

mechanism utilising the microphyllous condition as an aid under conditions of water-stress being kept in sight. The cabinets were set at near-saturation (98%–100% r.h.) and at a medium low level (45%–50% r.h.). Fluorescent tubes provided a light intensity of 400 f.c. with a 14 hour day-length, while a day temperature of 15°C and night temperature of 10°C were applied.

After four weeks definite differences began to appear between the plants undergoing the two treatments (Fig. 2). At high humidities the petioles were longer, making the habit much more loose and extended, than at low

FIG. 2—Contrasting humidity experiment. Plants above were grown at 98%–100%, those below at 45%–50% r.h. Fluorescent tubes provided a light intensity of 400 f.c. with a 14 hr daylength. Day temperatures 15°C, night temperatures 10°C. High humidity treatment produced thinner cuticles, fewer hairs, larger laminae, and longer petioles than low humidity. No effects were produced in depth of lobing.

humidities, the higher level also producing much thinner laminae with poorly developed hairs. In the matter of leaf dissection however there was no significant difference. As a seasonal factor influencing variations in dissection, humidity must be ruled out at least at the levels applied.

In a second experiment a temperature contrast was provided, the upper level at 20°C, the lower at 10°C, both cabinets being lowered 5° at night. A humidity level of 90% was chosen, otherwise conditions were as in the first experiment. Again at the end of about four weeks differences of the same kind as were seen in the first experiment appeared in habit and surfaces of the leaves, but at the same time changes began to appear in the depth of the notches. At 20°C the leaves took on a relatively undivided form, similar to that of young plants, but at 10°C they became deeply three-lobed (Fig. 3). These differences were exactly parallel to those found in the wild so that it was concluded that temperature could at least be a contributing factor in the natural variation (see Fisher 1954).

FIG. 3—Contrasting temperature experiment. Larger plants received 20°C, smaller received 10°C. Lighting of 400 f.c. and humidities of 90% were provided. Higher temperatures produced shallower notches as well as thinner cuticles, fewer hairs, larger laminae, and longer petioles than low temperatures.

As humidity differences had no effect on leaf shape, it is reasonable to exclude transpiration as a temperature dependent mechanism likely to be involved in the leaf shape changes. Of the two other temperature-sensitive processes mentioned, photosynthesis and translocation, only the first is usually regarded as light dependent, at least below saturation levels.

If the temperature effect on leaf shape were the result of a limit on photosynthesis at lower temperatures, lowered light intensities should produce similar effects. A third experiment was therefore arranged to compare the effects of two contrasting light intensities, 200 and 800 f.c. Measurements on forest margins have shown that *R. hirtus* does not normally grow where the intensities are less than about 200 f.c. Accordingly this level of intensity was chosen as a lower limit although direct measurements of the light tolerance of the species had not been made. As in the earlier experiments, standard conditions of 14 hours daylength, 90% r.h. and 15°C with a 5° drop at night were given. No effects on leaf shape occurred although the effects previously observed on habit, leaf thickness and hair production were repeated.

POSSIBLE PHYSIOLOGICAL MECHANISMS

Although no conclusive results have been found with these experiments certain suggestive features occur. The absence of a low light effect leaves translocation rather than assimilation as a major mechanism likely to be involved in the temperature effect on leaf-shape. The absence of a humidity effect similarly points away from a mechanism involving transpiration.

While many physiological processes at the biochemical level are temperature dependent, the possibility of a purely physical process is not in any way precluded by the available evidence. The likelihood of processes involving differential diffusion rates in morphogenesis has been discussed by Turing (1952) and Melville (1957). It may be useful to examine some of the implications of a temperature-dependent supply rate mechanism in relation to the developmental histology of the leaf primordium.

In *Ranunculus hirtus* the leaf primordia exhibit an initial lobing into three parts during the first week after visible initiation (Fig. 4). From a knowledge of the plastochron, determined autoradiographically (Fisher

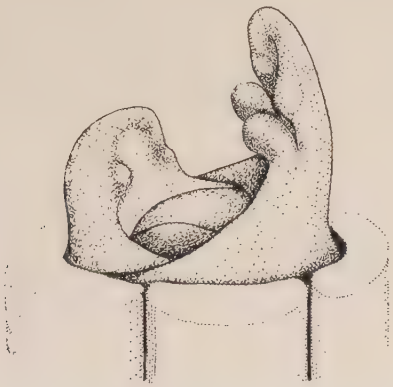

FIG. 4—*Ranunculus hirtus*. Sketch of stem apex showing meristem and three leaf primordia. Primordium on left is approximately 8 days old.

1956) and from the number of affected leaves after a low temperature treatment, the sensitive stage has also been estimated to occur within the first two weeks. Longitudinal sections provide a more precise estimate, recognisable differences between treated and untreated plants first being visible in sections cut 10 days after the beginning of treatment. The primordia showing these effects are between 2 and 3 plastochrons from the apex, hence with a plastochron of approximately 5 days, the effects appear in primordia 10 to 15 days from inception. Such primordia would therefore be between 0 and 5 days old at the beginning of treatment. The changes produced may be summed up in the following way. At lower temperatures extra notches or clefts appear at an early stage around the primordial margin so that additional lobes are initiated. At higher temperatures these clefts or notches are filled out by more evenly distributed meristematic divisions around the primordial margin, so that lobing in the mature leaf so initiated is much reduced.

Early in the life of any primordium, differential orientation of the cell divisions leads to the formation of procambial tissues, distinguishable from the surrounding tissues by the shape and position of the cells. It is not until

a considerably later stage that thickening and lignification of the simpler xylem elements marks the beginning of the extensive qualitative differentiation of the mature tissues. Even at the earlier stages however, the conducting efficiency of the elongated cells of the procambium is likely to be greater than that of the randomly arranged cells around them. In terms of resistance to diffusion it is clear that there is a smaller number of cross walls in a given length of procambium than in an equivalent length of promesophyll. Given such variations in the resistance to movement of substances diffusing out from the base of the primordium to the rapidly dividing cells of the margin, it is clear that some parts of the margin are likely to be favoured at the expense of other parts if through any cause the supply rates become limiting to growth. Those cells furthest from the most efficient channel would be the most affected if the rate were to drop, as a result of reduced temperature for example. Were the supply rates not limiting, consumption at the ends of the more efficient channels would be incomplete and any surplus could then move laterally to make up for any deficiencies at the ends of the less efficient channels.

The above argument would appear to hold whether temperatures operate directly on the various rates of movement in different zones of the primordium or on the production or supply of a particular limiting substance. Such substances might be simple growth materials such as carbohydrates or even water, or more complex substances such as amino-acids or even auxins. Leaf-development hormones have long been postulated by Went (1943), and Bonner (Bonner and Haagen-Smit 1939) has presented some evidence for adenine in this role, but really conclusive evidence for a leaf-hormone has yet to be produced. In any case, whether biochemical or biophysical in origin, the effect of limited supply rates in the mechanism put forward here operates through preferential channelling of such materials through procambial tissues.

The implications of an electrokinetic mechanism in the phloem have been recently discussed by Fensom (1957, 1958) and by Spanner (1959). If such a mechanism were normal in more mature conducting tissues the possibility of its operation even in very young tissues must be considered. In the above discussion of distribution rates as a factor in morphogenesis, it was assumed that relatively simple diffusion processes were involved. If electro-kinetic processes were in operation different features should be evident. The first requirement for active-transport is the provision of electrically polarised membranes which, through the medium of highly mobile ions such as potassium, act as pumps connecting the source of materials with the site of their consumption. Whereas in the phloem two kinds of cell occur side by side, each with a particular role in the transport system suggested by Spanner, no such differentiation is apparent in the primordial tissues. There is therefore no immediate reason why the cross walls of promesophyll tissue should act any differently from those in procambium if either were kept polarised by an active re-circulation of charged particles. However, as there could be a higher frequency of such polarised membrane pumps in promesophyll than in procambium, active transport in the promesophyll could be a considerably more significant component of total movement that it would be in the procambium.

In order to reconcile these considerations with the results observed in *Ranunculus hirtus*, in particular the apparent inhibition of development between the veins at lower temperatures, it would appear necessary that the metabolic component involving electro-osmosis should have a significantly higher temperature coefficient than the ordinary diffusion component. As far as the electro-osmotic step in the metabolic component is concerned however, the temperature coefficient would be identical with that for diffusion, as both processes are limited by viscosity relations determined by inter-molecular forces. Nevertheless, the possibility of an active uptake step (as opposed to an electro-osmotic one), being more important in promesophyll than in procambium, still leaves a way open for a greater temperature dependence of transport processes in promesophyll. The temperature coefficient (Q_{10}) of such active-uptake stages is in the order of 2 to 5 (Collander, 1959) whereas that of ordinary diffusion processes is approximately 1·2 (Höber, 1947) thus providing the required differential response that would lead to preferential channelling of growth materials through the procambium at lower temperatures.

Tracer Experiments

Using $^{14}CO_2$, some preliminary experiments to follow the distribution of assimilates into the tissues of the leaf primordia have been carried out. The isotope was administered in the light from a small glass capsule sealed to the undersurface of a mature leaf with porometer wax (see Thaine and Walters, 1955) and an interval of two hours allowed for assimilation before freeze-drying of the apex was commenced. Embedding was carried out in the vacuum finger of the freeze-drying apparatus using an infrared lamp to melt a small quantity of degassed paraffin after drying for sixteen hours. Sections cut at 10μ were brushed flat on to freshly dried plates of Ilford G5 nuclear emulsion, an exposure of fourteen days in a shielded dark-box being given. After development, the wax of the sections was removed with solvent and the slides examined under absolute ethanol.

Although definite tracks were present, there was no differentiation apparent between promesophyll and procambial zones of the primordium. It is probable that 2 hours is too long an interval to allow for clear differentiation in the apex. It will be necessary to prepare a series of apices at intervals from a few seconds up to perhaps 30 minutes, in order to find the time after administration of maximum differentiation between the tissues, if any such differentiation occurs. These experiments will be reported in a subsequent paper.

ECOLOGICAL SIGNIFICANCE OF LEAF-SHAPE PLASTICITY IN *Ranunculus hirtus*

In microphyllous leaves, restriction of the mesophyll to narrow zones surrounding the major veins is believed in many cases to permit water to move more readily into the surrounding tissues. In large leaves, particularly those not finely dissected, outer marginal portions may be some distance from major veins and hence may suffer damage under conditions of excessive transpiration.

In *Ranunculus hirtus,* finely dissected leaf forms are produced in cooler seasons when water stresses might be expected to be least. However, on the cool montane-forest margins favoured by *R. hirtus,* exposed situations may be colder even in summer than more sheltered ones, and yet be exposed to the fast-drying conditions of rapid air-movement, high light intensity, and low humidity. Within the sheltered margin, conditions are usually warmer while at the same time, moist, shaded, and still. This combination of conditions allows a possible explanation to be put forward for the existence of plastic leaf forms in *R. hirtus* under the control of temperature.

It is conceivable that the change in leaf shape adjusts the leaf form to conditions which accompany the temperature change, rather than to the temperature change itself. By such means, the habitat range and seasonal tolerance of the species might be extended. It is interesting to note that such a response would adapt the species only to the peculiar conditions of a forest margin where rising temperatures tend to be accompanied by reduced moisture stresses. The reverse situation is the more common one in most environments, and warmer conditions in these places, by reducing leaf dissection, might well place *Ranunculus hirtus* at a selective disadvantage under the increased transpiration stresses that would arise (Gregor, 1956).

It should follow from this hypothesis that, at any time, the leaf shapes found should be more dissected in open situations than in more sheltered situations. Any genotypic variation in ability to respond should lead to a greater preponderance of relatively more adaptable individuals in places where relatively greater variation in stress takes place. This should occur, if the hypothesis is justified, not only for populations in different climatic regions, but also, in clinal form across the forest margin, more adaptable forms tending to accumulate towards the more exposed end of the local ecological gradient. At the regional level, evidence suggesting such genotypic variation has been found, plants from more northern populations showing less response under the experimental conditions than those from further south. As yet, no variation has been observed in the plasticity responses within any particular population, but its existence between populations makes intrapopulational variation a strong possibility.

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SEASONAL BEHAVIOUR OF SOME MONTANE PLANT SPECIES

By D. Scott,* Botany Department, University of Otago.

(Received for publication, 1 July 1960)

Summary

The dates of flowering, fruiting, and seeding of 83 plant species during the 1958–59 season are given. These are principally of the montane tussock grassland of the Godley Valley, Lake Tekapo.

Two thirds of the species flower in November and December. There is some evidence that some species had a second flowering period in the autumn.

Of the 20 species that occurred throughout the altitudinal range studied 4 showed about a 2 week lag in phenological events at the higher altitudes (c. 5,000–6,000 ft) as compared with low altitudes (c. 2,500 ft). Also 5 species showed a 1–2 week lag on south aspects as compared with north aspects of similar altitude. This is probably related to temperature.

Introduction

Knowledge of the seasonal behaviour of plant species (phenology) is essential in assessing the effect of grazing animals on the range land vegetation of New Zealand, for it is only by taking such knowledge into account that assessment can be made of the effect of seasonal change in grazing pressure on the life cycle of each of the species. Accordingly, preliminary data are presented here of the time of flowering, fruiting, and seeding of plant species in the tussock grassland and related vegetation in the Godley Valley, Lake Tekapo (N.Z.).

Methods

Observations were made during the 1958–59 season near nineteen temporary climate stations on north, east, and south aspects at altitudes from 2,000 to 6,000 ft in the Godley Valley. Adjacent to each climate station, three 4 ft × 4 ft quadrats were pegged out and when the climate stations were being serviced at 2–4 weekly intervals notes were made of the commencement of growth, flowering, fruiting, and seeding of the species in these quadrats. On each occasion it took two or three days to visit all stations.

Results

Results are given in Table 1, where the following abbreviations have been used.

Section	Symbol	
Aspect	N,E,S,	= North, East, and South aspect respectively
Altitude	L	= Low altitude, between approximately 3,500 and 2,100 ft (valley bottom)
	M	= Mid altitude 3,500–4,500 ft
	H	= High altitude above 4,500 ft

*Present address: C/o Botany Department, Duke University, North Carolina.

Section	Symbol	
Phenology	fl(j)	= Just flowering, i.e., many flower buds with a few flowers
	fl	= Flowering
	fr	= Fruiting, i.e., period between fertilisation and commencement of seed shed (In cryptogams this refers to the development of the sporophyte or sporangiophore)
	s	= Seed shed in flowering plants or spore discharge in cryptogams.
	s(o)	= Seeding mostly over
Remarks	I	= Records incomplete, i.e., records from less than three stations
	A	= Indication of an autumn flowering period
	N	= Apparently no difference in phenology for a wide ranging species at different sites
	Dl	= A lag of about 2 weeks in phenological events at high altitudes in a wide ranging species
	Da	= A lag of about 1–2 weeks on south aspects as compared with north aspects of similar altitudes

The species have been arranged into eight groups based on the time of commencement of flowering. The different species flower in succession from early October through to late January with two-thirds of them flowering during November and December.

Most plants were showing new spring growth when they were visited in early October. The exceptions were the moss species, which were showing some new growth when visited in late August.

Of the twenty wide-ranging species with sufficient records, four show a lag of about 2 weeks at the high altitudes, and five show a lag of about 2 weeks on south aspects as compared with north aspects at similar altitudes. This lag is probably related to temperature as the march in temperatures during one year at different altitudes and aspects in the same location showed a 2–4 week lag in reaching successive temperature levels during the period September to January (Fig. 1).

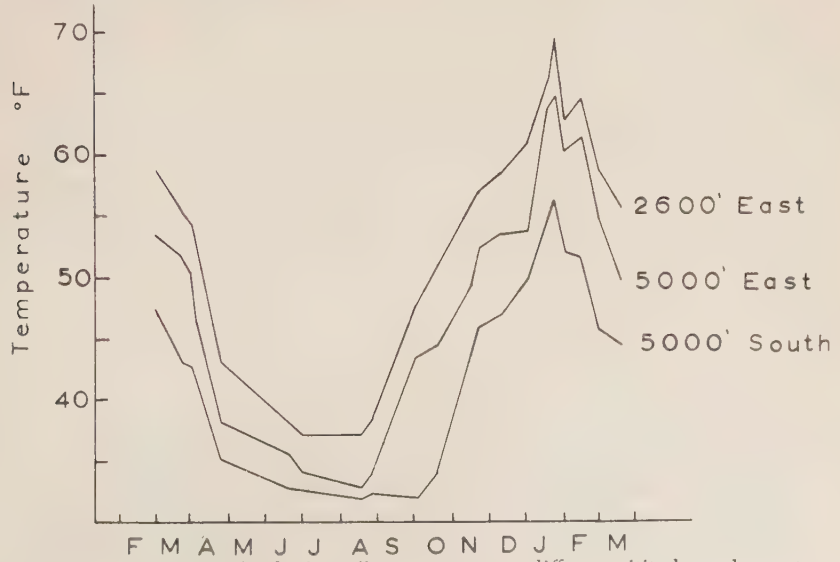

FIG. 1—Seasonal march of 8 in. soil temperatures at different altitudes and aspects.

TABLE 1—Phenology of Montane Species

Species	Ecology		Date of Observation												Remarks
	Aspect	Altitude	22–24/6	18–21/8	1–3/10	16–18/11	10–11/12	31/12–2/1	18–19/1	2–4/2	16–18/2	2–3/3	19–21/3	24–25/4	
(a) Species beginning to flower in October															
Moss spp.	N,E,S	H,M,L			fr,s	fr,s	fr,s	fr,s							
(b) Species beginning to flower early in November															
Aira caryophyllea	N,E	M,L				fl	fr	fr,s	s(o)						
Anthoxanthum odoratum	N,E	M,L				fl	fl,fr	fr	fr,s	s	s	s(o)			
Carex colensoi	N,E	H,M,L				fl	fl	fl,fr	fr	fr	fr,s	s	s		N
Colobanthus strictus	N,E	H,M,L				fl	fr,s	s(o)							I Rapid seed shed
Leucopogon fraseri	N,E	H,M,L				fl	fl,fr	fr	fr	fr	fr	fr	fr,fl	fr	N
Luzula campestris	N,E,S	H,M,L				fl	fl,fr	fr,s	s	s	s(o)				N
L. ulophylla	E	L				fl	fr	s	s	s(o)					I
Oreomyrrhis andicola	N,E	H,M,L				fl	fr	fr	fr	fr,s	fr,s	s			N
Pterostylis mutica	N,E	M,L				fl	fl,fr	fr,s	s	s(o)					I
Ranunculus gracilipes	S	H,M,L				fl	fl,fr	fr,s	s						I
R. lappaceus var. *multiscapus*	N,E	H,M,L				fl	fl,fr	fr,s	s	s(o)					N
Viola cunninghamii	N,E	H,M,L				fl	fl,fr	s(o)							I
(c) Species beginning to flower late in November															
Angelica decipiens	N,S	H				fl(j)	fl,fr	fl,fr	fr,s	fr,s	s	s(o)			I
Brachycome sinclairii	N,E,S	H				fl(j)	fl	fl,fr	fr	fr,s	s(o)	s,fl			A N
Cerastium caespitosum	N,E	M,L				fl(j)	fl,fr	s	s(o)			fl			A
Crepis capillaris	N,E	L				fl(j)	fl	fl	fl,fr	fl,fr	fl,fr,s	fl,fr,s	fl,fr,s	fl,fr,s	Long flowering season
" "	N,E	H						fl	fl	fl,fr	fl,fr	fl,fr,s	fl,fr,s	fl,fr,s	Dl
Discaria toumatou	N,E	L				fl(j)	fl,fr	fr	fr,s	s					I
Dracophyllum kirkii	S	M,L				fl(j)	fl			fr					I
Drapetes dieffenbachii	S,E	H				fl(j)	fl	fr	fr		fr,s	s	s(o)		I
Helichrysum bellidioides	N,E	M,L				fl(j)	fl	fl	fl,fr	s	s(o)				I
Myosotis pygmaea	N,E	M,L				fl(j)	fl	fr,s	s	s(o)					I
Poa colensoi	N,E	M,L				fl(j)	fl	fl	fl,fr	fr,s	s	s	s	s	Dl
" "	S,N,E	H					fl(j)	fl	fl,fr	fl,fr	fr,s	fr,s	s	s	Da
P. lindsayi	N,E	M,H				fl(j)	fl	fl	fl,fr	fr,s	s	s(o)			I
Rumex acetosella	N,E	L				fl(j)	fl	fl,fr	fl,fr,s	fl,fr,s	fr,s	s(o)			
	S	H					fl(j)	fl	fl,fr	fl,fr,s					

(d) Species beginning to flower early in December

Aciphylla aurea	N,E	M,L				fl			s	s				I
A. monroi	N,E,S	H,M				fl	fl,fr		fr					I
Anisotome aromatica	S	H,M				fl	fl,fr	fr	fr,s	s	s(o)			
var. *lanuginosum*														
Celmisia gracilenta	E,N	M	fr		fr	fl	fl,fr	fl,fr	fl,fr,s	fr,s	s	s(o)		Dl
	E,N	H				fl(j)	fl	fl,fr	fl,fr	fr,s	s	s(o)		
Coprosma petriei	E,N	M,L	fr		fr	fl?	fr			fr	fr	fr	fr	I Long fruiting season
Cotula perpusilla	E	L				fl	fr				s(o)			I
Craspedia uniflora	N,E	M,L				fl	fl	fl,fr	fl,fr,s	s	s(o)			I
Dracophyllum pronum	S	H				fl	fl	fr	fr,s	fr,s	s	s(o)		
Gaultheria rupestris	E	M				fl	fl,fr	fr	fr	fr	fr	s	s(o)	I
Geranium sessiliflorum	N,E	H,M,L				fl	fl,fr	s	s	s(o)				N
Gnaphalium collinum	N,E	H,M,L				fl	fl	fr,s	fr,s	s(o)				I
G. luteo-album	N,E	H,M,L				fl	fl,fr	fl,fr,s	fr,s	s(o)		fl		I R
Hydrocotyle novae-zealandiae	N,E	M,L				fl	fr				s			I
Hypochaeris radicata	N,E	H,M,L				fl	fl	fl,fr	fl,fr,s	fr,s	fr,s	s, fl	fl	N A
Lagenophora cuneata	N,E	M,L				fl	fl	fl,fr	fr,s	fr,s	s	s(o)		I
Linum catharticum	N,E	L				fl	fl	fr,s	s(o)					I
Ophioglossum coriaceum	N,E	H,M				fr		fr,s	s(o)					I
Prasophyllum colensoi	N,E	M,L				fl	fl,fr	fl,fr,s	fr,s	s	s(o)			I
Raoulia australis	N,E	H,M,L				fl	fl,fr	fl,fr	fl,fr,s	fl,fr,s	s(o)			Dl I
R. grandiflora	S	H				fl	fl	fl	s	s(o)				I
Senecio lagopus	N,E	M				fl(j)	fl,fr	fl,fr	fr	s	s	s(o)		Da
	S	M,H					fl(j)	fl	fr	fr,s				
Stellaria gracilenta	N,S	M,H				fl	fl	fl						I
Trifolium arvense	N,E	L				fl	fl	fl	fl,fr	fr,s	s	fl		I R
T. dubium	N,E	L				fl	fl	fl,fr	fr					I
T. repens	N,E	M,L				fl	fl	fl	fl,fr	fl,fr	fr,s	s	s	I

(e) Species beginning to flower late in December

Acaena anserinaefolia	N,E	H,M,L				fl(j)	fl	fl,fr,s	fr,s	s	s	s		N
Cassinia vauvilliersii	E,S	M,L				fl(j)	fl	fl	fl	fl,fr	fl,fr	s	s	I
Celmisia coriacea	E,S	H,M				fl(j)	fl	fl,fr	fr	s	s	s	s	I
C. lyallii	S,E	H,M				fl(j)	fl,fr	fr	s	s	s(o)			I
Epilobium chloraefolium or *erubescens*	N,E	H,M,L				fl(j)	fl,fr	fl,fr,s	fr,s	s	s(o)			Da
	S	M					fl	fl,fr	fr,s	fr,s	s			
Festuca novae-zealandiae	N,E	L				fl(j)	fl	fl,fr	s	s	s(o)			
	N,E	H					fl	fl	fl,fr,s	s	s	s(o)		Dl
	S	H					fl(j)	fl	fl,fr	fl,fr	s	s	s	Da
Holcus lanatus	N,E	L				fl(j)	fl	fl	fr	fr,s	fr,s	s	fl	I R
Lycopodium fastigiatum	N,E	H,M				fr(j)	fr	fr	fr	fr	s	s		
Lycopodium fastigiatum	S	H,M					fr(j)	fr	fr	fr	s	s		Da
Microseris scapigera	N,E	M,L					fl	fl,fr	fr	fr,s	s			I
Scleranthus uniflorus	N,E	M,L					fl	fl,fr	fl,fr	fr	fr,s	fr,s	s	
Wahlenbergia albomarginata	N,E,S	L,M				fl(j)	fl	fl,fr	fl,fr	fl,fr,s	fr,s	fr,s		
	N,E	H					fl(j)	fl	fl	fl,fr,s	fl,s	fr,s	fl,fr,s	Dl

TABLE 1—Phenology of Montane Species—*continued*

Species	Ecology		Date of Observation												Remarks
	Aspect	Altitude	22–24/6	18–21/8	1–3/10	16–18/11	10–11/12	31/12–2/1	18–19/1	2–4/2	16–18/2	2–3/3	19–21/3	24–25/4	
(f) Species beginning to flower early in January															
Agropyrum scabrum	N,E	H,M,L						fl(j)	fl	fr	fr,s	fr,s	s	s,fl	A N
Blechnum penna-marina	N,E,S	H,M,L						fr(j)	fr	fr,s	s	s	s	s	I
Celmisia novae-zealandiae	S	H						fl(j)	fl	fl,fr	fl,fr	fl,fr,s	fr,s	s	I
Centaurium umbellatum	N,E	L							fl	fl,fr	fl,fr,s	fr,s	fr,s		
Dianthus armeria	N,E	L							fl	fl,fr	fr,s	fr,s	s,fl	fl	R I
Danthonia flavescens	N,E,S	H,M						fl(j)	fl	fr	fr,s	s	s(o)		
Dichelachne crinita	N,E	M,L							?	fr	fr	s			I
Euphrasia laingii	S,E	H,M						fl(j)	fl	fl	fl,fr	fl,fr	fl,fr,s		
Forstera tenella	S	M							fl,fr	fr	fr				I
Helichrysum filicaule	N,E,S	M,L						fl(j)	fl	fl,fr,s	fl,fr,s	fr,s	s(o)		I
Poa caespotisa	N,E	M,L							fl	fr,s	s	s	s	s	I
Raoulia glabra	N,E	M,H						fl(j)	fl	fl,fr	fl,fr,s	fl,fr,s	s	s	
Thelymitra longifolia	N,E	M,L						fl(j)	fr	fr,s	s	s(o)			I
Viltadinia australis	N,E	L						fl(j)	fl	fr	fr,s	fr,s	fr,s,fl		A
(g) Species beginning to flower late in January															
Agrostis ?tenuis	N,E,S	H,M,L							fl(j)	fl	fr	fr,s	fr,s	s	N
Danthonia setifolia	N,E	H,M							fl	fl,fr	fr,s	s			I
Deyeuxia forsteri	N,E	H,M,L							fl	fl	fr	fr,s			I
Dracophyllum uniflorum	S	M							fl(j)	fl	fr	fr	s		I
Gentiana serotina	N,E	M,L							fl	fl	fl,fr	fr	s	s	I
Koelervia sp.	N,E	H,M,L							fl	fl,fr	fr,s	s			I
Senecio haastii	S	H							fl	fl					I
(h) Species with long flowering season															
Pimelea prostrata	N,E	H,M,L	fl	fl		fl	fl,fr	fr,s	fr,s				fl		Flowering principally in November

Nine species showed some flowering in the autumn though this was never as profuse as their main flowering period earlier in the season.

While these are only preliminary results they should assist further investigation directed towards extending knowledge to more species; more precise determination of time of phenological events; variation between sites; variation between years; and the climatic factors responsible.

Specimens of all species listed have been placed in the Otago University Herbarium.

ACKNOWLEDGMENTS

Thanks are expressed to Professor G. T. S. Baylis for his advice. The work was carried out under a N.Z. Wool Board Scholarship.

A NOTE ON THE STATISTICS OF RELATIONSHIP USED IN A RECENT REPORT OF THE NEW ZEALAND COUNCIL FOR EDUCATIONAL RESEARCH

By F. Cockrem, Massey Agricultural College, Palmerston North.

(Received for publication, 9 May 1960)

Summary

The statistics of relationship used by Parkyn in a recent report of the N.Z. Council for Educational Research are briefly discussed and some inconsistencies and errors in his use of tau_c are pointed out.

Introduction

In a recent study "Success and Failure at the University Part I" (Parkyn 1959) use was made of Kendall's (1955) tau coefficient to measure the degree of relationship between entrance standards and performance at the first year of the university course. Because tau_c is rarely used, and as the conclusions of this study depend upon it to a considerable extent, some discussion of this coefficient is of interest.

The Properties of Tau_c

Tau_c was derived by Stuart (1953) to meet the case where a measure of strength of association ranging between $+1$ and -1 was required for data in the form of a contingency table. It was derived from the tau_a and tau_c rank correlation coefficients of Kendall (1955). One important result of its method of derivation was that, for the maximum value of ± 1 to be achieved, all observations must lie on the longest diagonal of the table and *"that the frequencies in these cells are equal or as nearly equal as possible"*.

The second of these requirements means that the distribution required for the independent variable is less likely of attainment in most data than the normal distribution required for the product moment correlation.

For a bivariate normal distribution tau_a can be converted to the correlation coefficient (Schaeffer and Levitt 1956) and tau_a also bears a simple relationship to tau_c because of the method of derivation used by Stuart (1953).

Tau_c in Parkyn's (1959) Study

The effect on this study of the method of derivation of tau_c has been examined. The independent variables were 'normalised', a usual procedure for validating tests which are to be applied to a complete population. However, in this case 'normalising' will have had the effect of making the attainable value of tau_c less than one, as the 'normalised' distribution will have been far removed from Stuart's (1953) requirement of equal frequencies.

Table 1 gives the maximum possible values of tau_c in some of Parkyn's tables, using the actual distribution of the independent variable and assuming that the resulting frequencies fell only on the diagonal.

TABLE 1—The Maximum Values Attainable by Tau_c in Some of Parkyn's Tables

Table Number (Parkyn's References)	Actual Tau_c	Maximum Possible Tau_c
5.27	0.17	0.86
5.38	0.35	0.91
5.42	0.20	0.98
5.48	0.26	0.97
5.51	0.35	0.86
5.62	0.21	0.86

Some theoretical possibilities have also been considered and a perfect (equal frequency) and three other distributions along the diagonal are shown in Table 2 with the calculated value of tau_c and the percentage of students which could be eliminated without error if university grades D and E were considered undesirable. It can be seen that a perfect correlation can exist for selection purposes with a value of tau_c as low as 0·6, although it should be noted that such an extreme distribution does not occur in Parkyn's data.

TABLE 2—Theoretical Contingency Tables with the Values of Tau_c for Different Distributions of 50 Students

Distribution	1					2					3					4				
Grades University	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
School																				
A	10					2					2					2				
B		10					8					4					3			
C			10					18					31					35		
D				10					15					10					7	
E					10					7					3					3
Tau_c	1.0					0.92					0.71					0.60				
% Students Grades D & E	40%					44%					26%					20%				

It appears that the use of tau_c as a non-parametric method is liable to lead to greater difficulties than might arise from using the standard techniques developed for normal distributions. Analysis of variance at least can stand a lot of abuse with regard to the departure of data from a normal distribution (Box 1953). Regression techniques might have been better for this data as the independent variable was selected but this can be judged only from examination of the original non-normalised data. Such techniques have been found satisfactory for similar problems on the validation of Officer Selection Boards in Britain (Reeve 1949).

The interpretation of the values of tau_c obtained by Parkyn is further complicated by the method used of converting to the product moment correlation and then using the value of this coefficient for interpretation. The formula of Schaeffer and Levitt (1956) is applicable only for a bivariate normal distribution. As alleged non-normality was the reason for the use of tau_c it seems that this formula ($r = \sin 90$ tau) was not applicable, or that, alternatively, a product moment correlation could have been calculated directly.

Such a use of a product-moment correlation would not have been possible with the non-normal data and there might have been computational advantages in the use of one measure of relationship for all the cases studied. The validity of this argument rests on the ability of the measure chosen to give equivalent estimates of the relationship for all such cases. Because of the different types of distribution tau may not do this which thus reduces its value as a universal measure.

DISCUSSION

It is possible that the use of tau did not introduce important errors into Parkyn's estimates of the degrees of relationship. The point is that not only is this not known without a more precise knowledge of the properties of tau but also that the final conclusions rest on the "actual degree of relationship" (Parkyn 1959, p. 11).

However, the final interpretation of the results of an investigation of the type discussed here must include subjective elements as well as a knowledge of the measured degrees of relationship. For example, conclusions will depend on which relationships were selected for measurement and which of many possible interpretations are made if a low correlation is found. It is hoped to discuss matters of this sort elsewhere (Cockrem, in press), the purpose of this note being to point out some of the technical limitations of the methods used and the consequent difficulties of interpretation.

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NOTE IN REPLY

By G. W. PARKYN

(Received for publication, 16 November 1960)

In the interest of the majority of those who would need a report upon my study of university performance in New Zealand (Parkyn, 1959) it seemed to me desirable to use some index of the relationship between the many variables involved. By so doing, the general trends of a large number of two-way distribution tables could more readily be perceived and kept in mind. For the same reason it seemed desirable to use just one kind of index throughout the study.

The main difficulties involved in choosing a suitable index were carefully considered at the beginning of my investigation, and, after discussing the problems with many people both in New Zealand and overseas, I came to the conclusion that, on balance, the tau coefficient was rather more suitable than any other for the purposes of this study.

Dr Cockrem's note (Cockrem, 1960) gives an interesting and useful theoretical analysis of some of the difficulties, but while I recognise these, I do not think that they have in fact substantially affected the general conclusions of the particular study in question, for the basic data of the investigation are provided in the contingency tables, and are, of course, unaffected by whatever subsequent indices of relationship are calculated from them. Likewise, the general conclusions I have drawn can be substantiated by reference to the basic tables without using any index of relationship (though this is a cumbersome and tedious method). Having done both I consider that the use of the tau coefficient is not likely to be the source of any misinterpretation of the data of this investigation.

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CORRIGENDA

N.Z. J. Sci. 2: 533

Table 1, column 1.

for *Viola lyalli* read *Viola lyallii*

Ibid. 2: 535

for *H. classifolia* read *H. crassifolia*

Ibid. 2: 537

for *Viola lyalli* read *Viola lyallii*

Ibid. 3: 37

For the paragraph following the regression equation subsititute:

$\hat{O}$ is the predicted sample count of Oithona at any given value of t, s, p, n; $\bar{o}$ is the mean sample count of Oithona; $t = 10\ (T-\bar{t})$; $S = 10\ (S-\bar{s})$; $p = P/\bar{p}$; $n = N/\bar{n}$; where T is the sample temperature in degrees centigrade; S is the sample salinity in $^0/_{00}$; P is the sample count of *Paracalanus*; N is the sample count of *Eminius nauplii*; $\bar{t}$, $\bar{s}$, $\bar{p}$, $\bar{n}$ are the means corresponding to T, S, P, N.

Ibid. 3: 107

3rd line from foot of page

for over 10 months females

read over 33 months females

Ibid 3: 113

Table 10, last column

for Increase of Males over Females

read % Increase of Males over Females

Ibid. 3: 297

In line 12,

for 8°C read − 8°C

ADDENDUM

Ibid. 3: 508

A further reference relative to the subject matter of this paper, and not available up to the time of going to press is:

BORSOS, O. 1957: Experimentelle Morphologische Beobachtungen in der Gewebestruktur der Blätter von Ungarländischen Festuca-Arten. *Acta Bot. Acad. Sci. Hungaricae 3*: 219–42.

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